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METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF ISONIAZID IN TABLET DOSAGE FORM BY UV VISIBLE SPECTROSCOPY

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Abstract:

Tuberculosis (TB) is the world's second most common cause of death from infectious disease, next to human immunodeficiency virus (HIV). TB has become an increasing health problem since emergence of HIV; TB/HIV co-infection adds further complications in treating the disease. It is also a major concern for industrialized nations because of emergence of drug resistance, alcohol / drug abuse, growth of immigrants and other factors. Method employs formation and solving of simultaneous equation using 263 nm and 292 nm as two analytical wavelengths for both drugs in Distilled water. Isoniazid and at their respective λmax 263 nm and 292 nm shows linearity in a concentration range of 1-11µg /mL and 5-30µg /ml. Recovery studies for Isoniazid 98% and 102% for in case of simultaneous equation method confirming the accuracy of the proposed method. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific.

Keywords: *UV - Visible Spectroscopy, Isoniazid, Distilled water.*

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INTRODUCTION:

Name of the drug : **Isoniazid**

Chemical Structure

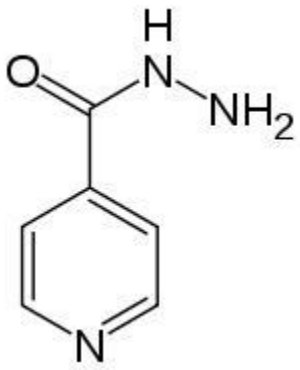

Figure: 1. Shows structure of Isoniazid

Brand Name : Solonex

Company Name : Macleods Pharmaceutical Pvt. Ltd.

Category : Antibiotic

Molecular Formula : $C_6H_7N_3O$

Molecular Weight : 137.1 g/mol

Physical Properties : While to off white – crystalline power

P_H Value : 5.5 to 6.5

P_{Ka} Value : 1.82

Protein binding : 91%

Half Life : 13.8 Hrs

Melting Point : 171.4 ^{oC}

Boiliing Point : 40 ^{oC}

Solubility : Soluble in organic solvent such as ethanol, DMSO, di-methyl formamide which should be purged with an inert gas, sparingly in aqueous buffer.

Mechanism of Action : Bactericidal; Especially for rapidly dividing cells. Affects mycolic acid (cell wall) synthesis. Inclusion of isoniazid in the regimen of patients with strain W MDR-TB was also associated with improved outcomes.

Dosage from and strength

Adult Dose : 4-6mg/kg/day

Route of administration : Oral, IV or IM.

Preparation: 50 mg, 100 mg or 300 mg scored or unscored tablets; 50 mg/5 ml oral suspension in sorbitol; solution for injection is 100 mg/ml. When given IV, dilute in 25 ml normal saline and infuse as a slow bolus over 5 minutes. Since compatibility information is not available, do not infuse "piggyback" with other drugs through a shared IV line.
Storage: Suspension must be kept at room temperature (15–25 °C) 30 ^{0C}

Uses: Use in pregnancy/breastfeeding: Safe during pregnancy; safe during
breastfeeding (both baby and mother should receive pyridoxine supplementation). Up to 20% of the infant therapeutic dose will be passed on to the baby in the breast milk. Use in renal disease: No dose adjustment for renal failure, but pyridoxine supplementation should be used. Use in hepatic disease: May exacerbate liver failure. Use with caution. Drug Interactions: Isoniazid is a CYP3A4 inhibitor. Isoniazid may increase the concentrations of certain cytochrome P450 enzyme substrates, including phenytoin and carbamazepine.
FDA Approval of Drug : Isoniazid is an antibacterial prescription medicine approved by the US Food & Drug Administration (FDA) for the prevention and treatment of tuberculosis (TB) is an opportunistic infection of HIV

Adverse reactions : Hepatitis (age-related).
Peripheral neuropathy.
Hypersensitivity reactions.
Other reactions, including optic neuritis, arthralgias, CNS changes, druginduced lupus, diarrhoea, and cramping with liquid product.

Contraindications : Patients with high-level isoniazid resistance who have failed an isoniazid-containing regimen should not receive isoniazid. History of allergic reaction to isoniazid.

Drug Interactions : Monitor concentrations of phenytoin or carbamazepine in patients receiving those drugs (increases phenytoin concentrations and risk of hepatotoxicity with carbamazepine), especially when undergoing isoniazid monotherapy. Rifampin tends to lower concentrations of these drugs and balance the effect of isoniazid.

Monitoring : Clinical monitoring of all patients on isoniazid is essential. Routine laboratory monitoring is not recommended for patients receiving isoniazid monotherapy for latent TB infection. For patients receiving multiple TB drugs or other hepatotoxic drugs, or with underlying liver disease (including viral hepatitis), baseline liver function testing is recommended. Follow-up liver function testing is determined by baseline concerns and symptoms of hepatotoxicity.

Alerting symptoms : Instruct patients to inform their health care provider right away if Any of the following occurs:

- Loss of appetite for a few days that does not go away
- Tiredness, weakness
- Moderate stomach pain, nausea or vomiting
- Numbness, pain or tingling of your fingers or toes
- Blurred vision, eye pain
- Yellow skin or eyes or dark-colored urine.
-

MATERIALS AND METHODS:

Chemicals and Reagents: Methanol, Ethanol, Acetonitrile, Choloform, Distilled water
Instruments:

SHIMADZU UV-1601 UV – Vis spectrophotometer, Detectors **(UV DETECTOR, ANALYTICAL),** Electronic Balance **(CITIZEN BALANCE BL-2204, SHIMADZU),** Sonicator (**ANALYTICAL),**

Reagents and Solutions

Diluent preparation : Take 300mg of Isoniazid and add 100ml of water. Dissolve and make up to 100ml in a volumetric flask.

Standard preparation: Accurately weigh300mg of isoniazid and transfer in to 100ml volumetric flask. Add about 10ml of solvent mixture sonicate to

dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Transfer 3ml of above solution in to a 10ml volumetric flask and make up the volume with diluent.

Sample preparation: Accurately weight 300mg of isoniazid and transfer in to 100ml volumetric flask. Add about 10ml of solvent mixture sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Transfer 3ml of above solution in to a 10ml volumetric flask and make up the volume with diluent.

Optimized chromatographic conditions

Wavelength - 263nm

Solvent - distilled water

Wavelength Selection

The standard solutions are prepares by transferring the standard drug in a selected solvent and finally diluting with the same solvent or Diluent. That prepared solution is scanned in the UV visible wavelength range of 200-400nm.This has been performed to know the maxima of Isoniazid. While scanning the Isoniazid solution we observed the maxima at 263 nm. The visible spectrum has been recorded on (SHIMADZU UV-1601 make UV – Vis spectrophotometer model UV-1601.The scanned visible spectrum is attached in the following page. The λ_{max} of the Isoniazid was found to be 263 nm in diluents as solvent system.

RESULTS AND DISCUSSION:

Development and Optimization of the Method

Proper wave length selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of Isoniazid, the analytical conditions were selected after testing the different physical properties.

Our preliminary trials using different solvents:

Table:1 Shows Solubility Study of Isoniazid

Trial no	Solvent	Result
1	Distilled Water	Soluble
2	Methanol	Completely soluble
3	Acetonitrile	Sparingly soluble
4	Ethanol	Sparingly soluble
5	Chloroform	Soluble

Selection of wavelength

The standard stock solution was scanned from 200-400nm and the absorption spectra were recorded at 263nm (ʎ1) and 292nm (ʎ2) respectively

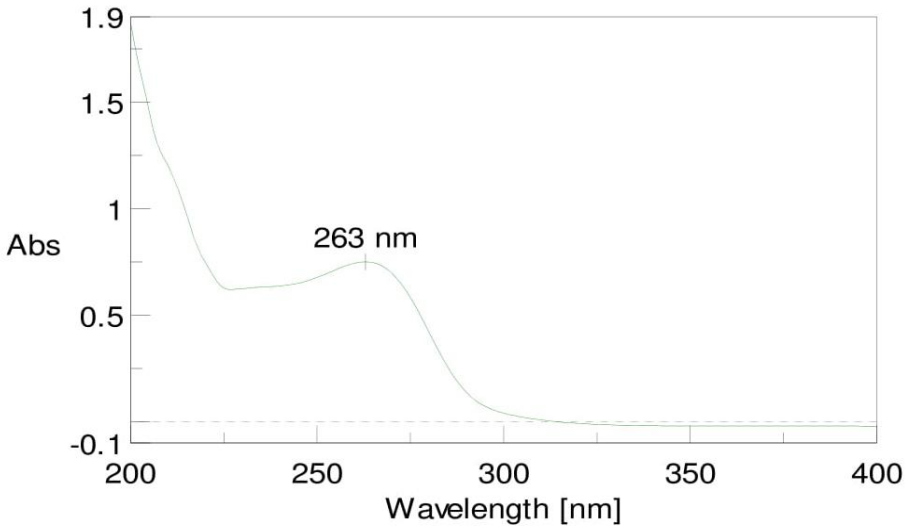

Figure: 2. Shows UV spectrum of Isoniazid

Table-2: Shows Assay Results of Marketed Formulations

Formulations	Actual concentration of Isoniazid (mg)	Amount obtained of Isoniazid %	% Isoniazid
Isoniazid	300 mg	99.90%	99.00%

Method validation:

1.Accuracy

Table: 3. Shows % Accuracy Recovery of Isoniazid

Lable claim	Amount Taken (mg)	Amount found(mg)	SD	C.OV	SE
80%	8 mg	8.03 mg	0.3	1.3	0.05
100%	10mg	9.95 mg	0.09	1.2	0.05
120%	12mg	11.79 mg	0.8	1.1	0.004

SD :- Standard Devilation, COV:- Coefficient of viaricene , SE:- Standard Error

Result: The accuracy for the average of triplicate in each concentration samples are within the limit.

Table: 4. Shows % Recovery of Isoniazid

Amount added (mg/Tb)	Amount found (mg/Tb)	Average % recovery
300mg	11.79 mg	99.00

PRECISION:

Intermediate precision (inter-day and intraday precision):

Intermediate precision of the method was checked by assay the sample solution on same day at an interval of one hour (intraday precision) for three hours and on three different days (interday precision) the result was reported in This study indicates that the solutions can be analyzed within 48-72 h without having any bad effect on chemical stability of the drug in presence of urea. The results of the same were.

Table: 5. Shows Precision Results of Isoniazid

Parameters	Drug	Labe Claim	Amount Taken	Amount Found	S.D	C.O.V	S.E
Precision (Inter – day)	Isoniazid	300mg	10 mg	9.88mg	0.02	1.1	0.02
Precision (Intra – day)	Isoniazid	300 mg	10 mg	9.75 mg	0.05	1.2	0.07
Accuracy	Isoniazid	80%	80 mg	8.03	0.3	1.3	0.05
		100%	10 mg	9.95	0.09	1.2	0.05
		120 %	12 mg	11.79	0.8	1.1	0.04

SD :- Standard Devilation, COV:- Coefficient of viaricene , SE:- Standard Error

Result:

The precision values are found within the limit

Acceptance criteria: A method is said to be precise if the % RSD is < 2 %, the results show % RSD for the intermediate precision as 0.67-1.49 which are within the limits and hence the method is said to be precise.

Acceptance criteria: A method is said to be precise if the % RSD is < 2 %, the results show % RSD for the intermediate precision within the limits and hence the method is said to be precise.

LINEARITY AND RANGE:

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of INH and Vit B6. For simultaneous equation method the Beer- Lambert's conc. range was found to be for5- 25µg/ml for INH and 5-25µg/ml. The results were reported.

LINEARITY OF ISONIAZID:

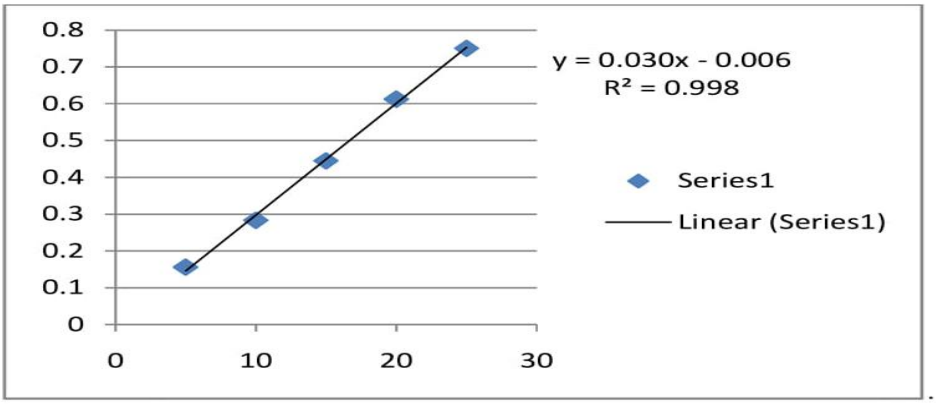

Figure:3. Shows Calibration curve of Isoniazid

Table:6 . Shows linearity results of Isoniazid

S.no	Linerty level	Concentration	Area
1	I	10µg	0.1
2	II	20 µg	0.3
3	III	30 µg	0.45
4	IV	40 µg	0.6
5	V	50 µg	0.75
Correlation Coefficient	-	-	0.9998
Intercept	-	-	Y=0.030X- 0.006
Slope	-	-	0.0304

Result:

The overlain spectra of INH and VitB6 exhibit λ max of 263 nm and 292 nm respectively which are quite separated from each other. Standardcalibration curves for INH and VitB6 were linear with correlation coefficients (r) values in the range of 0.998- 0.999 at all the selected wavelengths and the values were average of six readings with standard deviation in the range of . 0.02-0.8.. The accuracy of the method was confirmed by recovery studies from tablet at three different levels of 80 % , 100 %, 120 % .The most striking feature of this method is its simplicity, economy and rapidity, non- requiring consuming sample preparations such as extraction of solvents, heating, degassing which are needed for UV procedure. These are new and novel methods and can be employed for routine analysis in quality control analysis..

Method Robustness:

Robustness of the method was determined by carrying out the analysis under different Wavelength i.e. at 263nm, 292nm . The respective absorbances of 10µg/ml were noted SD < 2%) the developed UV-Spectroscopic method for the analysis of Pyridoxine (API).

RUGGEDNESS:

In the ruggedness study, the influence of small, deliberate variations of the analytical parameters on the absorbance of the drug was examined. The factor selected was a change in the analyst. The Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 5- 25µg/ml was noted. The result was indicated as %RSD.

Table-7: Results showing Ruggedness for Isoniazid
Analyst -1

Concentration (µg/ml)	Absorbance of Isoniazid
30	0.613
30	0.599
30	0.610
30	0.612
30	0.613
30	0.622
Mean	**0.6115**
SD	**0.8**
%RSD	**1.20**

Table-8: Results showing Ruggedness for Isoniazid
Analyst -2

Concentration (µg/ml)	Absorbance of Isoniazid
30	0.615
30	0.613
30	0.607
30	0.617
30	0.610
30	0.617
Mean	**0.613166**
SD	**0.8**
%RSD	**0.655**

RESULT:

The result of ruggedness study of the developed assay method was established in Table. The result shown that during all variance conditions, assay value of the standard solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as ruggedness.

LOD & LOQ : The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected **,** The LOD are calculated from the calibration curve by formula LOD = 3.3 x SD/ b The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision, The LOQ are calculated from the calibration curve by formula LOQ = 10 x SD/ b

Table: 9. Shows LOD & LOQ results of Isoniazid

Parameters	Isoniazid
LOD	0.481 µg/ml
LOQ	1.59 µg/ml

VALIDATION PARAMETER RESULTS:

Table: 10. Shows summary of validation parameter Results

S.NO	Parameter	Acceptance criteria	UV
1	% recovery	98-102	99.00%
2	Linearity range (µg/ml)	-	5- 25(µg/ml)
3	Correlation Coefficient	NLT 0.9998	0.9998
4	Precision	%RSD(NMT 2%)	0.02
5	Intermediate Precision	%RSD(NMT 2%)	0.05
6	Ruggedness	%RSD(NMT 2%)	0.655
7	LOD	-	0.481(µg/ml)
8	LOQ	-	1.59(µg/ml)

CONCLUSION:

From the experimental studies it can be concluded that first UV-Spectroscopic method is developed for Isoniazid in marketed pharmaceutical dosage form. The developed method for the drug (Isoniazid) was found to be accurate and precise.

The great features of spectrophotometric methods are their simplicity, economical and rapidity. The results of method validation showing that the developed analytical procedure is suitable for its intended purpose and meets the Guidelines given by the ICH.

The developed method was successfully applied for the routine analysis of Isoniazid in bulk and pharmaceutical dosage form in the future.

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