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Original Article

Development and Validation of Isoniazid in Bulk and Pharmaceutical Dosage Forms by UFLC Method

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ABSTRACT

In the present research work a new simple, specific, precise and accurate Ultra-fast liquid chromatographic method was developed and validated for estimation of Isoniazid in bulk and marketed pharmaceutical dosage forms. **Experimental Approach:** The method was developed by using BDS Hypersil C-8 column (5µm, 250 mm x 4.6 mm) as stationary phase and Milli Q water: Methanol (95:05%v/v) as mobile phase. The flow rate of the mobile phase was 1 ml/min. The analysis was performed at ambient temperature with UV detection at 290nm. The retention time of Isoniazid was found to be 6.8 minute. The run time of analysis was 10 minutes with injection volume of about 20 µL. Developed method was validated as per ICH Q2 guidelines using linearity and range, specificity, selectivity, precision, accuracy, robustness, ruggedness, limit of detection and limit of quantification. **Findings and Discussion:** The drug showed linear response between the concentration ranges from 50-800 µg/ml. Method was found to be selective, specific and precise with % RSD less than 2%. The % drug recovery was found to be within the acceptance limit of 90-110%. **Conclusion:** The developed UFLC method can be used for the routine quality control of Isoniazid in bulk and dosage form.

Keywords: Isoniazid, Ultra-fast, UV-Detection, ICH Guidelines, Quality Control.

1. INTRODUCTION

Tuberculosis is a chronic granulomatous disease and a major health problem in the developing countries and mainly caused by the bacteria "*Mycobacterium tuberculosis''*. Many first and second line drugs were reported for the effective treatment of tubercular infection. Out of this drugs Isoniazid is the one of the excellent first line drug used for effective treatment[1]. Chemically Isoniazid is pyridine-4-carbohydrazid also known as isonicotinic acid hydrazide occurs as colorless, odourless, crystalline powder. It acts by inhibiting the synthesis of mycolic acid in the mycobacterium species.[2]

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For active tuberculosis it is often used together with rifampicin, pyrazinamide, and either streptomycin or ethambutol.[3] It is available in the form of injections and tablets and administered by oral, intramuscular and intravenous routes.[4] Few titrimetric methods[5] which includes assay of isoniazid with perchloric acid in non-aqueous medium[6], redox reaction based spectrophotometric assay of isoniazid[7] and Chromatographic[8] methods were reported for estimation of Isoniazid in bulk and pharmaceutical dosage forms also the use of molecular line probe assay for the detection of resistance to isoniazid and rifampicin have been reported by WHO.[9]But no scientific record was reported for assay of isoniazid by UFLC method. Hence, in the present research work attempt has been made to develop and validate new UFLC method.

2. MATERIALS AND METHOD

Chemicals and Reagents:

All the chemicals used for analysis was pure and analytical grade obtained from the store house of KLE College of Pharmacy, Belagavi. HPLC grade water was obtained from Millipore water purification system. HPLC grade methanol was procured from SDFCL.

Apparatus and Instrumentation:

Analysis was performed using Shimadzu UFLC connected with prominence UV/Vis detector SPD-20A and isocratic prominence liquid chromatogram pump LC-20AD with manual injection system. For the data acquisition, monitoring and processing output LC solution software was used. For weighing of drug sensitive analytical balance of Sartorius was used. UV-Spectrophotometer of Shimadzu was used for selection of wavelength for analysis.

Preparations of Mobile phase:

The mobile phase for the present study used was composed of HPLC water: Methanol in the ratio of 95:05 %v/v. The prepared solvent system was filtered through vacuum filtration assembly using 0.22 µfilters and sonicated for 15 minutes.

Preparation of stock solution and serial dilutions:

100 mg of Isoniazid was weighed and transferred into 100 mL of volumetric flask and dissolved in few mL of mobile phase, sonicated and volume was made upto the mark to obtained 1000 µg /mL of drug. From the above stock solution serial dilutions were made to obtain 50, 100, 200, 400 and 800 µg /mL of drug solution.

Development of UFLC method:

Isoniazid is easily soluble in water hence water was chosen as aqueous phase and for the good resolution of peak methanol was used as organic phase. Few trials were made by using different solvent system but good resolution and better chromatogram was obtained by using Milli Q water: Methanol (95:05 %v/v) as mobile phase and BDS Hypersil C-8 column as stationary phase.

Selection of wavelength for detection:

Isoniazid was dissolved in few mL of mobile phase and solution was scanned between the 400-200 nm range using UV-Spectrophotometer. The drug showed maximum absorbance at 290nm. Hence it was selected as wavelength of detection for UFLC analysis.

Determination of retention time:

50 mcg/mL of Isoniazid solution were injected manually into the UFLC at a flow rate of mobile phase 1mL/minute using syringe with injection volume of about 20 µL. The UV detection was performed at 290nm with run time of about 10 minutes. By using above optimized parameters chromatogram was obtained with retention time of 6.8 minutes with minimum tailing factor and maximum theoretical plates.

Method validation:

The developed UFLC method was validated by using following typical parameters such as specificity, selectivity, linearity and range, precision, robustness, ruggedness, recovery, LOD and LOQ as per the ICH guidelines. [10,11,12]

System suitability:

In order to check the suitability of instrument for the planned analysis system suitability was performed on daily basis by injecting 100 µg/mL of drug solution.

Specificity and Selectivity:

20 µL of mobile phase solvent and 100 µg/mL drug solution was injected into the UFLC system to observe the interference at the retention time of analyte.

Linearity and Range:

Serial dilution of drug prepared ranging from 50-800 µg/mL and injected into the UFLC in triplicate with injection volume of 20 µL. Chromatograms were integrated and data was collected. Calibration curve was constructed by using mean peak area of analyte *vs* concentration and coefficient of determinants and linear regression were calculated.

Precision:

It was performed in terms of system precision, intraday and interday precision. System precision was performed by injecting six replicates of 20 µL of 200 µg/mL of drug solution into UFLC and area was calculated from chromatograms obtained and % Relative Standard Deviation (RSD) was calculated. Intraday precision was performed by injecting six replicates of 200 µg/mL of drug solution into UFLC at two different times in a day independently, chromatograms were obtained and % RSD for peak area was calculated. Interday precision was performed by injecting six replicates of 200 µg/mL of drug solution into UFLC on two different days independently, chromatograms were obtained and % RSD for peak area was calculated.

Robustness:

The robustness of method was performed by changing small deliberate change in the composition of mobile phase and comparing the data from the previously obtained chromatograms. For the robustness study, following are the two different mobile phase compositions were used and six

replicates of drug solutions were injected using the a*)*Composition-I: Milli Q water: Methanol (96:04 %v/v) and ***b)***Composition-II: Milli Q water: Methanol (94:06 %v/v). Chromatograms obtained and % RSD of peak area was calculated.

Ruggedness:

It was performed by different analyst on different day, by injecting six replicates of drug solution. Chromatograms obtained and %RSD for peak area was calculated.

Limit of Detection and Limit of Quantification:

Limit of Detection (LOD) and Limit of Quantification (LOQ) values was obtained from the statistical methods and reported.

Recovery:

It was performed at three different levels (80%, 100% and 120%) by standard addition and sample addition method. Three replicates at each level were injected and area was obtained and amount of drug recovered was calculated.

Application of developed UFLC method for estimation of Isoniazid in marketed formulation:

Tablets formulation containing 300 mg of Isoniazid dose was obtained from the local market of Belgaum. 20 tablets were weighed and triturated to make fine powder. Powder equivalent to 300 mg of Isoniazid were weighed and transferred into 100 mL of volumetric flask, dissolved in sufficient amount of mobile phase and sonicated for 10 minutes and volume was made upto the mark using the same to obtained 3000 µg/ml of drug solution. The above solution was filtered and 10 mL of filtrate was taken and transferred into 100 mL of volumetric flask to get 300 µg/ml of Isoniazid and injected into UFLC and drug content was calculated.

3. RESULTS AND DISCUSSION

Method development:

The method development was started with solubility analysis of isoniazid in different solvents. The literature review and practical analysis revealed that the analyte is freely soluble in water. Hence Milli-Q water was used in more amounts in the mobile phase composition. Several trials were made by using different solvent composition utilizing acetonitrile and methanol. Mixture of acetonitrile and water gives the poor elution of analyte and hence methanol was used to obtained the intense and sharp peak with minimum tailing.

Selection of wavelength of detection of analyte:

The solution containing isoniazid was scanned in the UV-Spectrophotometer between the range of 400-200 nm and spectrum was obtained. The drug showed maximum absorbance at 290 nm and detection was carried out at 290nm. The UV spectrum was showed in Figure 2.

Determination of retention time:

20 µL of 200 µg/ml drug solution was injected into UFLC and chromatogram was obtained. The retention time of drug was found to be at 6.9 minute as showed in Figure 3. The developed method parameters were presented in Table 1.

System suitability and carry over test:

It was performed on daily basis prior to carry out analysis to check the instrument performance. Three replicates of analyte solution and one blank solution were injected and % RSD obtained for the retention time, peak area, tailing factor and theoretical plates. The results obtained showed the %RSD for retention time and peak area was less than 2%, tailing factor was less than 2 and plate counts was more than 2000 and also blank sample not showed any peak area at the retention time of analyte, which proves the no carry over in the developed method. The data of system suitability was showed in Table 2.

Method validation

Specificity and selectivity:

Developed method was found to be specific and selective as there is no interference of any peak at the retention time of isoniazid.

Linearity and Range:

The method was found to be linear between the concentration range of 50-800 mcg/mL with correlation coefficient 0.9998 and % curve fitting 99.98. The data obtained was presented in Table 3. Calibration curve was constructed and showed in Figure 3.

Precision:

The % RSD of area obtained in each replicates were calculated for system precision, intraday and interday precision was found to be less than 2% and hence developed method was found to be precise. The results were presented in Table 4.

Robustness and Ruggedness:

For the robustness parameter deliberately altered chromatographic conditions showed no significant impact on developed method parameters. As they showed no change in the RT of analyte and % RSD of peak area obtained was 0.45 % and 1.87% for composition 1 and composition 2 respectively hence the developed method was found to be robust.The % RSD of peak area obtained from chromatograms of different analyst was found to be 0.36 % and hence method was found to be rugged and the results were presented in Table**5.**

LOD and LOQ:

The LOD and LOQ values of Isoniazid were found to be 13.92µg/ml and 42.19µg/ml respectively by statistical calculations.

Recovery:

The % drug recovery at each level was found to be within the acceptance of 90%-110%. Recovery data was presented in Table 5. The report of method validation parameter was presented in Table 6.

Drug content estimation of Isoniazid in marketed formulation by UFLC:

Three replicates of sample solution were injected into the UFLC and data obtained from chromatograms % drug content in Isoniazid tablet was calculated. The amount of

drug present in the marketed tablet formulation was found to be 2.97 mg and % assay was found to be 99.11 %

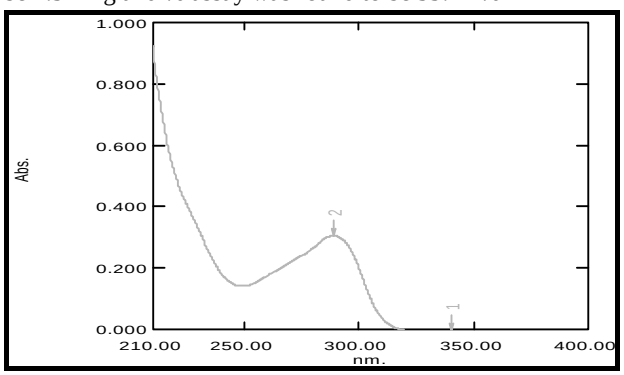

Fig 1: UV-Spectrum of Isoniazid

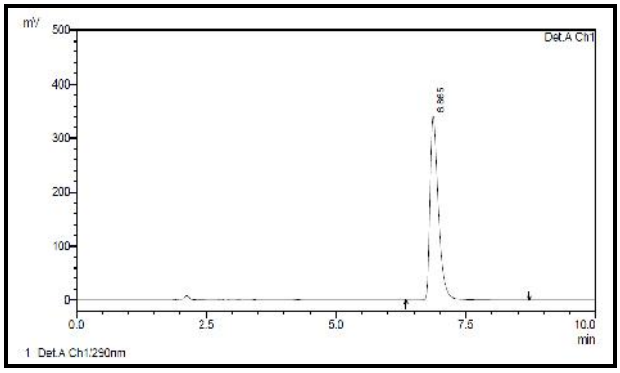

Fig 1: Chromatogram of Isoniazid

Table 1: Developed method parameters

Instrument name	**UFLC**
Make	Shimadzu
Stationary phase	BDS Hypersil C-8 column
Mobile phase	Milli Q water: Methanol (95:05 % v/v)
Flow rate	1 mL/Minute
wavelength	290 nm
Injection volume	20 uL
Run time	10 minutes
Retention time	6.8 minutes

Table 2: System suitability parameter

Replicates	Retention Time	Peak Area	Theoretical plates	Tailing factor
1	6.8 minute	452037	9297	1.36
2	6.9 minute	442935	9230	1.32
3	6.7 minute	443734	9154	1.35
%RSD	**1.47%**	**1.13%**	**0.78%**	**1.55%**

Table 3: Linearity and range data of Isoniazid

Sr. No.	Concentration	Peak Area	Statistical parameters	Values
1	50 µg/ml	447030	**Corr. coefficient**	0.9998
2	100 µg/ml	906335	**Slope**	8708
3	200 µg/ml	1788120	**% Curve fitting**	99.98
4	400 µg/ml	3576240	**LOD**	13.92 µg/ml
5	800 µg/ml	6981637	**LOQ**	42.19 µg/ml

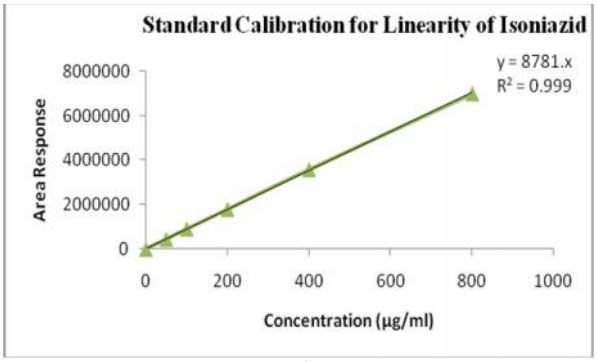

Fig 3: Standard calibration curve of Isoniazid

Table 4: Precision data of Isoniazid

Precision	System Precision	Intra-day Precision (1)	Intra-day Precision (2)	Inter Day-1Precision	InterDay-2Precision	InterDay-3Precision
Replicates	**Peak Area**	**Peak Area**	**Peak Area**	**Peak Area**	**Peak Area**	**Peak Area**
1	1900055	3734641	383940	6578803	6453809	6411849
2	1910000	3741224	373014	6595358	6556809	6534209
3	1860588	3743361	383940	6670752	6553309	6481149
4	1846956	3744261	384590	6570520	6456839	6434989
5	1898431	3742261	389876	6569883	6651849	6621649
6	1878784	3735616	384283	6579830	6554889	6504689
%RSD	**1.32%**	**0.11%**	**1.44%**	**0.58%**	**1.13%**	**1.15%**

Table 5: Robustness and Ruggedness data of Isoniazid

Robustness	Mobile phase 1	Mobile phase II	Ruggedness
Replicates	**Peak Area**	**Peak Area**	**Peak Area**
1	449734	445239	1825813
2	447122	459734	1826831
3	444235	440122	1823861
4	444335	451235	1819831
5	446239	444037	1835621
6	446234	436239	1836391
%RSD	**0.455**	**1.87%**	**0.36%**

Table 6: Recovery data of Isoniazid

Levels	Conc. of Standard added	Conc. of Sample added	Total Conc.	Mean area obtained	Sample conc. obtained	% Drug recovery
80%	30µg/ml	50µg/ml	80µg/ml	754582	49.94 µg/ml	99.88%
100%	50µg/ml	50µg/ml	100µg/ml	906017	49.96 µg/ml	99.92%
120%	70µg/ml	50µg/ml	120µg/ml	1104836	51.90 µg/ml	103.8%

Table 7: Method validation report of Isoniazid

Validation parameter	Results obtained
Linearity and range	50-800 µg/ml
Specificity and selectivity	No interference of peak at retention time of analyte
System precision	Less than 2 %
Intraday precision	Less than 2 %
Interday precision	Less than 2 %
Robustness	Less than 2 %
Ruggedness	Less than 2 %
Limit of detection	13.92 µg/ml

Limit of quantification	42.19 µg/ml
Recovery	97-100%
Drug assay	99.11%

4. CONCLUSION

New UFLC analytical technique was developed for the estimation of isoniazid in bulk and validated as per the ICH guidelines. All the results obtained was found to be within the acceptance limit of the guidelines also developed method was found to be simple, selective, specific, precise, accurate, robust and rugged and can be used for the routine quality control of drug in bulk and dosage forms.

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