Validation of Isoniazid Analysis Method in 2 Fixed-Dosed Combination Dispersible Tablet by High Performance Liquid Chromatography

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ABSTRACT

Tuberculosis (TB) is a severe disease that causes significant morbidity and mortality globally, particularly in children. For children in advanced stages, combined drug therapy with isoniazid and rifampicin (two-fixed drug combination (2 FDC)) is recommended. The dissolving test requirements for producing two FDC dispersible tablets, however, are not listed in the Indonesian Pharmacopoeia or any other standard book. Because the only method of analysis presently in use is the ratio of the outcomes of the rifampicin and isoniazid dissolutions in the manufacturing of capsules, new methods of analysis must be developed and validated in order to make dispersible tablets. The aim of this research is to develop analytical methods for determining isoniazid levels in two Fixed-Dosed Combination (FDC) dispersible tablet formulations using High Performance Liquid Chromatography (HPLC). The motion phase is applied to a C18 column (4.6 mm x 250 mm, 5 m). Water:Dapar Phosphate:Methanol (850:50:10) is delivered at a flow rate of 1.5 ml/min with UV-Vis detectors at 254 nm. The validation parameter test results show validation criteria based on linearity parameters and range with correlation coefficients (r) of 0.9991, LOD 0,00038724 mg/mL, and LOQ 0.00117346 mg/mL; accuracy tests show isoniazid% recovery of 99.51%, precision tests show %RSD of 0.32%, and recovery of 100.13%. The results of the selectivity tests show that this method is very selective, as no other drugs respond at the same time as isoniazid. Based on the validation parameters and the determination of the isoniazid level that fulfills the requirements, it was determined that the HPLC method may be used to determine the amount of isoniazid in the 2 FDC dispersible tablet formulation resulting from the dissolution test.

Key word: FDC, HPLC, Isoniazid, TBC, Validation Analysis Method

INTRODUCTION

Tuberculosis (TB) is one of the leading causes of morbidity and has the highest mortality rate in the world. This disease is directly contagious and caused by the bacteria Mycobacterium tuberculosis (1). By 2021, 10 countries collectively accounted for 75% of the global gap between estimated TB incidence and the number of newly diagnosed people with TB. The top five contributors are India, Indonesia, the Philippines, Pakistan, and Nigeria (24%, 13%, 10%, 6.6%, and 6.3%, respectively) (2). The number of TB cases discovered in Indonesia in 2021 was 443,235: 434,967 drug-sensitive TB cases and 8,268 drug-resistant TB cases (3). TB cases attack not only adults but also children. Indonesia is one of the countries in Southeast Asia with the highest incidence of TB in children from 2010 to 2018, ranging from 9.4% to 11%. TB cases in children can be treated either by the administration of isoniazid and rifampicin combination therapy for the advanced phase (4).

A two-fixed drug combination (FDC) is the use of two active drugs in one form of preparation. 2 FDC is a dispersible tablet preparation that contains rifampicin and isoniazid. One of the quality requirements for the preparation of dispersible tablets is the dissolution test. The Drug and Food Supervisory Authority in Indonesia determines dissolution test requirements using the Indonesian Pharmacopoeia or other standard books. Based on the monograph listed in Indonesian Pharmacopoeia Ed. VI (2020), the preparation of 2 FDC capsules containing the active substance rifampicin using UV-Vis spectroscopic photometry and isoniazid using HPLC. The other research that has been carried out is the development of methods of analysis of FDC preparation rifampicin, isoniazid, and pyrazinamide using HPLC densitometry by Prabowo et al., (2012) and the development by Nasution et al., (2020) of the method of spectrophotometry UV-Vis on the analysis of isoniazid and vitamin B6 in tablet preparation.

However, neither the Indonesian Pharmacopoeia Ed. VI (2020) nor any other standard book lists the dissolving test requirements to produce two FDC dispersible tablets (5). This does not relieve the pharmaceutical sector of its need to undertake dissolution tests on its products. Since the only method of analysis currently in use is the ratio of the outcomes of the rifampicin and isoniazid dissolutions in the preparation of capsules, it is necessary to develop and validate new methods of analysis to produce dispersible tablets. The validation of the analysis method aims to ensure that the method used to measure product characteristics consistently obtains accurate results and that the methods used are reliable.

METHODS

Development of Analytical Method

A source of literature used as a reference in research and development is Indonesia Pharmacopoeia Ed. (5). The method used refers to rifampicin and isoniazid capsules. The analysis method was developed against the columns used and the preparation of the test solution by comparing the sample solution and the phosphate buffer 1:1 from the previous preparation. The method of analysis was developed until effective instrument conditions were obtained for the test sample.

Validation of Analytical Method

Validation of analysis methods is carried out by testing several validation parameters, namely: Linearity, Range, Limit of detection (LOD), Limit of quantification (LOQ), Accuracy, Precision, Selectivity, and Robustness. The test methods are in accordance with those found in the Indonesian Pharmacopoeia Ed. VI.

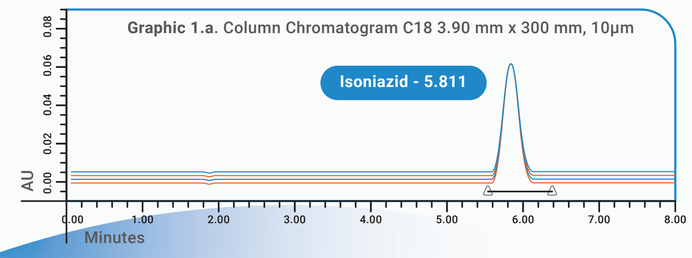
Determination of Sample Dissolution

Each flask in the dissolution tester was filled with 900 mL of 0.1 N HCl medium and the media temperature was set to 37°C ± 0.5%. Next, one tablet was put into each dissolution flask. Six baskets were dipped simultaneously into the dissolution medium, and the tool was turned on at a speed of 100 rpm. After 45 minutes, the solution was taken from each flask using a disposable syringe and put into a test tube. The solution was pipetted 1.0 mL each into a 20 mL flask and 1 mL of phosphate buffer was added. Then diluted with distilled water to the limit mark and shaken until homogeneous. Tolerance within 45 minutes of dissolving is not less than 80% (Q) of isoniazid. The dissolution test requirements at stage one are that each dosage unit is not less than Q + 5% (85%).

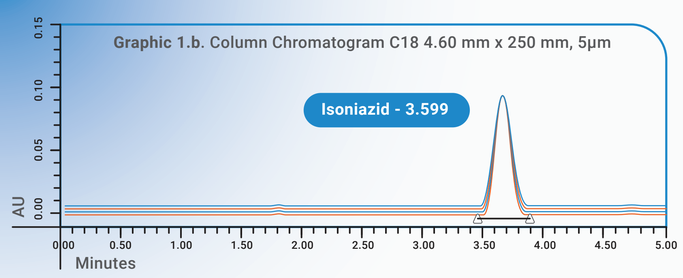
RESULTS

Instrumentation

HPLC with isocratic elution using column L1 (C18 4.60 mm x 250 mm, 5µm) and UV-Vis detectors operated at 254 nm wavelengths. Mobile phase comparison was used in the mixture of water:phosphate buffer:metanol (850:100:50) injected at a flow rate of 1.5 mL/min, and the solvents used are water, phosphate buffer, and HCl 0.1 N. The sample is injectable at a volume of 50 µl. The dissolution tester is used for the determination of isoniazid levels using a basket apparatus at a speed of 100 rpm within 45 minutes, and the dissolving medium used is 900 mL HCl 0.1 N.

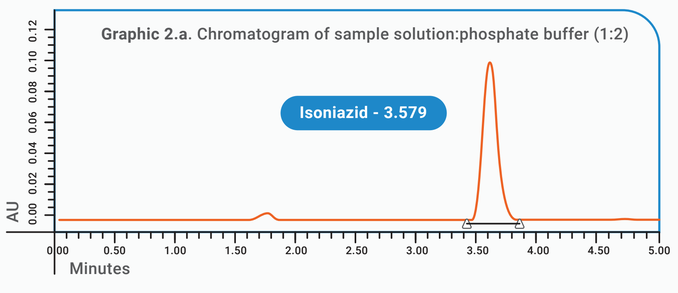


Graphic 1.a. Column Chromatogram C18 3.90 mm x 300 mm, 10µm

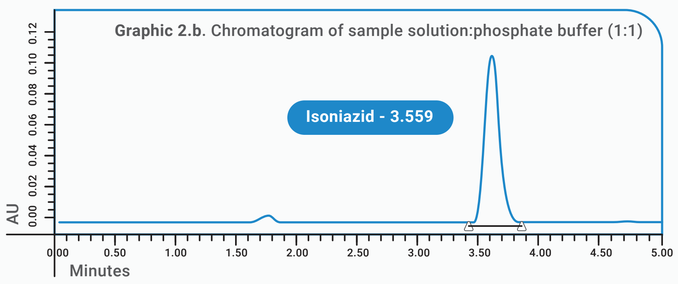


Graphic 1.b. Column Chromatogram C18 4.60 mm x 250 mm, 5µm

A faster retention time and sharper peak were obtained in the modified L1 column (Graphic 1b). The retention time obtained using the L1 column (C18 3.90 mm x 300 mm, 10µm) was 5.811 minutes, while using the L1 column (C18 4.60 mm x 250 mm, 5µm) was 3.599 minutes. The separation process occurs more quickly when using a modified L1 column. The use of smaller particles results in two main improvements in chromatographic separation, namely speed and increased separation (resolution). The increase in analysis speed comes from modifying smaller column dimensions so that active compounds are separated more quickly.



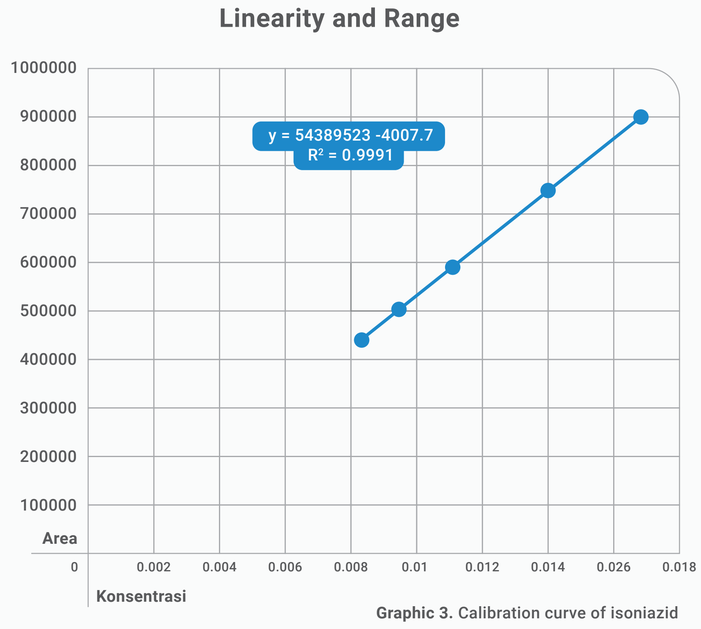
Graphic 2.a. Chromatogram of sample solution:phosphate buffer (1:2)



Graphic 2.b. Chromatogram of sample solution:phosphate buffer (1:1)

Based on Graphic 2, the use of a 1:2 phosphate buffer solution shows that the peak impurities are higher compared to the use of a 1:1 phosphate buffer solution. The addition of phosphate buffer functions to stabilize the pH. One factor that can be used to monitor the elution strength of the mobile phase is the pH value (9). Compounds that are acidic will be eluted with an acidic pH mobile phase, while compounds that are basic will be eluted with an alkaline mobile phase. The process of eluting the active substance requires the same pH between the mobile phase and the solution. The use of 1 mL of phosphate buffer is sufficient to support the analysis process, this also helps in the efficient use of solvents and reduces waste generated from the analysis process. Another reason is to use phosphate buffer with a ratio of 1 mL of test solution to 1 mL of phosphate buffer so that impurity peaks cannot be read on the chromatogram and the method can be more selective.

Validation of Analytical Method



Graphic 3. Calibration curve of isoniazid

Graphic 3 shows a linear relationship between isoniazid concentration and area. This shows that the higher the concentration, the higher the Area Under Curve (AUC). The linear line equation shows y = 54389523x – 4007.7 with a correlation coefficient (r) of 0.9991. The Relative Standard Deviation (RSD) obtained was 0.9087% with an average recovery of 99.64%. A correlation coefficient value close to one proves that the method used is good for sample measurements, so it can be concluded that the method used produces good linearity.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Testing the LOD and LOQ of an analyte can be seen from the standard deviation value of the response (σ) of the analyte being analyzed. The test results obtained a σ value of 6382.381, so the LOD obtained was at a value of 0.00038724 mg/mL. This means that at an analyte concentration of 0.00038724 mg/mL, it can still be detected by the system. The LOQ value obtained was 0.00117346 mg/mL. This means that a minimum analyte concentration of 0.00117346 mg/mL can be measured quantitatively with acceptable precision and accuracy.

Accuracy

The results of sample accuracy testing can be seen in Table 1, namely that the average recovery value obtained was 99.51% with a Relative Standard Deviation (RSD) of 0.68%. The recovery value at each concentration meets the requirements because the method used is said to have an adequate level of accuracy if it meets the accuracy requirements with a recovery value of 98–102%.

No	Consentration (%)	Sample (mg/mL)	Recovery (%)
1	60	0.008363	99.79
2	60	0.008363	100.97
3	60	0.008350	99.26
4	100	0.013898	99.51
5	100	0.013913	99.83
6	100	0.013908	99.51
7	120	0.016678	98.57
8	120	0.016683	99.14
9	120	0.016683	98.99
Average			99.51
RSD			0.68

Table 1 : Result of sample accuration

Precision

The test results are shown in Table 2. The analysis results have good precision, with an RSD value of 0.32% and an average recovery value of 100.79%. The method has good precision and meets the precision test requirements because none of the recovery values are outside the range of 98–120% and the RSD value obtained is ≤ 2%.

No	Consentration (%)	Sample (mg/mL)	Recovery (%)
1	100	0.013890	100.66
2	100	0.013915	101.19
3	100	0.013903	100.77
4	100	0.013898	100.63
5	100	0.013903	101.13
6	100	0.013903	100.32
Average			100.79
RSD			0.32

Table 2 : Result of sample presicion

Selectivity

The test results showed that the method was selective because there were no compounds that responded at the same time as the isoniazid retention time. This is because only the active substance isoniazid appears at the same time as the standard solution, while components such as placebo, distilled water, and buffer do not produce the same peak as isoniazid.

Robustness

Robustness is usually expressed as the absence of the influence of differences in operation or working environment on test results. The toughness test parameters are solution stability, mobile phase variations, and flow rate variations. In testing the stability of the standard solution and sample solution, it was carried out at different storage times. Sample solutions and standard solutions are stored at room temperature for 1 hour, 2 hours, 3 hours, 5 hours, and 7 hours. The standard solution has good stability and can be used safely for a period of 7 hours without experiencing significant changes. The test results of the sample solution can only be stable for up to 5 hours. 7 hours of storage influence the resulting area where the deviation exceeds the requirement limit of ≥ 2%. This shows that the sample solution has lower stability compared to the standard solution at the same time. Based on this, it is necessary to pay attention to the storage time of the sample solution to ensure the quality and accuracy of the analysis results.

Determination of Sample Dissolution

Based on the test, the minimum dissolution level was 94.63% with an average value of 99.06% and it was concluded that the isoniazid level in the dissolution test sample met the requirements because there was not one dosage unit whose value was less than 85% (Q + 5 %).

CONCLUSIONS

Development of an analytical method for determining the isoniazid content of samples from dissolution tests on 2 FDC dispersible tablet preparations using the HPLC method, using an L1 column (C18 4.60 mm x 250 mm, 5µm) with a ratio of sample solution and phosphate buffer (1:1). The results of the validation parameter testing provide values that meet the validation criteria based on linearity and range parameters with a correlation coefficient (r) 0.9991, LOD 0.00038724 mg/mL, and LOQ 0.00117346 mg/mL. The accuracy test produced a % recovery of isoniazid 99.51%, the precision test showed an RSD 0.32%, and a recovery 100.79%, the selectivity test results showed that this method was very selective because there were no other compounds that responded at the same time as the isoniazid retention time. The results of determining the isoniazid content in the average dissolution test sample were 99.06%. Based on the results of validation parameters and the determination of isoniazid levels that meet the requirements, it was concluded that the HPLC method can be used to determine isoniazid levels from dissolution tests in 2 FDC dispersible tablet preparations.

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