Journal of Science and Technology Research for Pharmacy
Volume 4 Issue 2 Year 2024 Pages 1-53

E-ISSN 2776-0685
https://journal.unnes.ac.id/journals/JSTRP/index

Validation Study of the Method of Analysis of Fe (II) Levels in Blood Addition Tablet Preparations by Wet Destruction and Dry Destruction Methods by UV-Vis Spectrophotometry

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Abstract: **Background:** Iron levels contained in blood supplement tablets need to be known by quality testing to ensure that the preparation contains the ingredients and the amount that has been determined. UV-Vis spectrophotometer is an alternative method for determining iron levels because it has lower operating costs and is simpler but has high sensitivity. The requirement for metal analysis using a UV-Vis spectrophotometer is that the sample must be in the form of a solution so that the deconstruction process needs to be carried out. Destruction is divided into two, namely dry and wet deconstruction. And validation of analytical methods including linearity test, LoD test, LoQ test, precision and accuracy. **Aim:** This study aims to compare two commonly used deconstruction methods, namely wet deconstruction and dry deconstruction in the determination of Fe (II) levels in blood supplement tablets. This study will also be carried out method validation which aims to demonstrate that the test procedures used obtain the expected results consistently and continuously. **Materials and Methods:** This research design uses a quantitative descriptive analysis approach with a pure experimental method designed to determine the iron content in blood supplement tablet samples by wet deconstruction and wet deconstruction by UV-Vis spectrophotometer instrument method. **Results:** Validation results include detection limit, quantitation limit test, accuracy test and precision test. Linearity is shown with R2 of 0.9986; detection limit of 1.10 mg/L; limit of quantitation of 3.62 mg/L; accuracy of dry deconstruction and wet deconstruction between 80-120%; and precision of dry deconstruction and wet deconstruction indicated by the percentage of RSD below 2%. Quantitative testing results show the percentage of blood tablet sample levels of wet deconstruction and dry deconstruction are 91.52%; 100.09%; 96.15%; 100.96%; 100.67%; and 93.39%; 108.29%; 99.47%; 93.47%; and 92.57%, respectively. **Conclusion:** Wet deconstruction and dry deconstruction methods can be used for the determination of Fe(II) levels in blood supplement tablets with valid and reliable results, according to the test parameters that have been carried out. Both methods showed consistent results with adequate accuracy and precision.

Keywords: wet destruction, dry destruction, uv-vis spectrophotometer of blood addition tablets, validation of analysis method

INTRODUCTION

Iron is one of the micro minerals needed in the human body. Iron in the human body has an important role in the formation of oxygen-rich red blood cells (Salman *et al*., 2018). One of the efforts in fulfilling iron needs can be done by consuming blood supplement tablets containing iron. So that by consuming blood supplement tablets can help increase hemoglobin levels in the blood (Sari *et al*., 2021).

Iron levels contained in blood supplement tablets need to be known by quality testing to ensure that the preparation contains the ingredients and the amount that has been determined. Determination of iron levels is generally carried out using the Atomic Absorption Spectrophotometer method. This method is known to have high sensitivity and selectivity, but requires relatively expensive operating costs, thus requiring other alternative methods (Marliana, 2019). Therefore, UV-Vis spectrophotometer is an alternative method for iron determination because it has lower operating costs and is simpler but has high sensitivity (Taufik *et al*., 2018).

Research using a UV-Vis spectrophotometer regarding the determination of iron levels in blood supplement tablet samples has been carried out by Kurniawati (2016), who prepares samples using wet deconstruction and dry deconstruction methods. Wulandari et al. (2019) said the results obtained in the study stated that Fe (II) levels with wet deconstruction method amounted to 99.9 mg and dry deconstruction amounted to 26.69 mg. Wet deconstruction produces more accurate and effective data in measuring iron levels in blood supplement tablets. However, the study has limitations because no validation of the analysis method has been carried out to ensure that the method used is able to produce accurate data. Therefore, it is necessary to conduct research on the comparison of validation methods for determining Fe (II) metal levels contained in blood tablets between dry and wet deconstruction.

METHODS

Materials and Methods

This research design uses a quantitative descriptive analysis approach with a pure experimental method designed to determine the iron content in blood supplement tablet samples by wet deconstruction and wet deconstruction with the UV-Vis spectrophotometer instrument method.

Time and Location

Samples of 5 different brands of blood supplement tablets were collected in Gunungpati District, Semarang City, Central Java. Then the metal content testing was carried out at the Chemistry Laboratory of the Chemistry Department, Faculty of Mathematics and Natural Sciences, Semarang State University.

Equipment and Materials

The tools used in this research are UV-Vis spectrophotometer, mortar and pestle, 50 mL beaker glass, 10 mL volumetric flask, 100 mL volumetric flask, 500 mL volumetric flask, stirring rod, 1 mL measuring pipette, 1 mL volumetric pipette, 2 mL volumetric pipette, 5 mL volumetric pipette, 10 mL measuring cup, 50 mL measuring cup, 250 mL erlenmeyer, stative and burette, micropipette, analytical balance, hote plate, oven, pH meter, whatman filter paper, funnel.

The materials used in this study were 5 samples of blood supplement tablets containing ferrous fumarate, distilled water, nitric acid (HNO3) p.a, HCl p.a, FeCl3.6H2O p.a, Na2S2O3.5H2O p.a, 1,10-phenanthroline p.a, sodium acetate p.a, acetic acid p.a.

Wet Destruction Sample Preparation

A total of 0.4 grams of sample was weighed and then put into a glass beaker. Add 5% HNO3 as much as 10 mL in a fume hood and heat on a hotplate for 1 hour until a few milliliters remain and the solution becomes clear. Then the solution was filtered and the filtrate was put into a 10 mL volumetric flask, added distilled water to the limit mark and homogenized.

Dry Destruction Sample Preparation

A total of 0.4 grams of sample was weighed and then put into a glass beaker. Next, the sample was heated in an oven at 110°C for 120 minutes. Then add HCl 6M as much as 10 mL into a glass beaker and heat on a hotplate for 1 hour until the ash dissolves. The solution was filtered and the filtrate was put into a 10 mL volumetric flask and added distilled water up to the mark.

Method Validation

Linearity

The linearity test is based on the coefficient of determination (R^2) on the linear regression equation of the methamphetamine calibration curve made from 5 concentration variations, namely 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm.

LOD and LOQ

The LoD and LoQ tests were determined by statistically calculating the linear regression equation of the methamphetamine calibration curve obtained from the linearity test results.

Precision

The accuracy of the method is checked and verified by repetition. Repetition is measuring each sample 6 times on the same day.

Accuracy

Accuracy testing was carried out by adding Fe standard solutions with concentrations of 2, 6, and 10 ppm to herbal medicine samples in a 1:1 ratio in cuvettes. The accuracy of the method was assessed based on the percentage of recovery.

Determination of Fe (II) UV-Vis Spectrophotometer Method

Preparation of 100 ppm Fe(II) Standard Solution

A total of 0.0483 grams of $FeCl_3$.$6H_2$ O crystals put into a glass beaker add a little aquadest to dissolve. Next, put it into a 100 Ml volumetric flask and add distilled water to the limit mark.

Preparation of 100 ppm Na2S2O3 Solution
A total of 0.0157 grams of Na2S2O3.5H2O is put into the baker and add a little aquadest to dissolve. Next, put it into a 100 Ml volumetric flask and add distilled water until the limit mark.

Preparation of 1000 ppm 1,10-phenanthroline Solution
A total of 0.025 grams of 1,10-phenanthroline solid was put into a 50 mL beaker and 10 mL of distilled water was added. The mixture was then heated on a hotplate with a temperature of 60°C until the entire solid dissolved. The solution was transferred into a 25 mL volumetric flask. then added distilled water until the limit mark.

Preparation of Acetate Buffer Solution pH 4.5
A total of 3.8554 grams of sodium acetate is put into a glass beaker and add a little water to dissolve. Put into a 100 mL volumetric flask, add 5 mL of acetic acid and add water until the limit mark. The solution is measured using a digitas pH meter until it shows a pH of 4.5.

Preparation of Blank Solution
The 100 ppm sodium thiosulfate solution was taken as much as 1.1 mL and put into a 100 mL volumetric flask. then add 1.5 mL of 1,10-phenanthroline 1000 ppm, add 1.5 mL of acetate buffer pH 4.5 and add 5 mL of acetone. Add Aquadest until the limit mark.

Determination of Maximum Wavelength
100 ppm Fe standard solution was put into a 10 mL volumetric flask as much as 0.5 mL, then added 1.1 mL of 100 ppm sodium thiosulfate solution. Furthermore, 1.5 mL of 1,10-phenanthroline 1000 ppm solution and 1.5 mL of acetate buffer Ph 4.5 were added. The mixture was added 5 mL of acetone and diluted using distilled water until the limit mark. The solution was shaken and allowed to stand for 120 minutes, then the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 450-560 nm.

Calibration Curve Creation
100 ppm Fe standard solution was put into a 10 mL volumetric flask as much as 0.1 mL, then added 1.1 mL of 100 ppm sodium thiosulfate solution. Furthermore, 1.5 mL of 1,10-phenanthroline 1000 ppm solution and 1.5 mL of acetate buffer pH 4.5 were added. After that, acetone was added to the mixture as much as 5 pH and diluted using distilled water until the limit mark. The solution was shaken and allowed to stand for 120 minutes, then the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength. This procedure was repeated 4 times with the amount of 100 ppm Fe standard solution as much as 0.2 mL; 0.3 mL; 0.4 mL; and 0.5 mL, respectively. The absorbance results obtained then made a calibration curve between absorbance with Fe concentration.

Determination of Fe(II) Level
Measurement for dry deconstruction The preparation solution was taken 0.1 mL and put into a 10 mL volumetric flask. Then added 1.1 mL of 100 ppm sodium thiosulfate and 1.5 mL of 1000 ppm 1,10-phenanthroline solution. To keep the pH acidic, 1.5 mL of acetate buffer pH was added, then 5 mL of acetone was added and diluted using distilled water until the limit mark. The solution was shaken and allowed to stand for 120 minutes, then the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength. Measurements were taken 6 times.
Measurement for wet deconstruction of the prepared solution was taken 0.1 mL and put into a 10 mL volumetric flask. Then added 1.1 mL of 100 ppm sodium thiosulfate and 1.5 mL of 1000 ppm 1,10-phenanthroline solution. To keep the pH acidic, 1.5 mL of acetate buffer pH 4.5 was added, then 5 mL of acetone was added and diluted using distilled water until the limit mark. The solution was shaken and allowed to stand for 120 minutes, then the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength. Measurements were taken 6 times.

Data Analysis
Sample levels are known based on the standard curve equation y = bx ± a, where y is the absorbance value and x is the measured level. From the reading of the sample, it is known that the absorbance as y and x is the measured level with the w/v (mg/L) level.

RESULT AND DISCUSSION

Determination of operating time was carried out using a standard solution with a concentration of 6 mg/L measured at a wavelength of 519 theoretically. From the operating time measurement, a stable measurement time

was obtained starting from 1 hour 12 minutes to 1 hour 14 minutes. In this time range, the absorbance obtained is consistent and stable with a value of 0.401 (table 1).

Table 1. Operating time result data

Time	Absorbance
1 hour 05 minutes	0,382
1 hour 06 minutes	0,381
1 hour 07 minutes	0,835
1 hour 08 minutes	0,388
1 hour 09 minutes	0,390
1 hour 10 minutes	0,395
1 hour 11 minutes	0,396
1 hour 12 minutes	0,401
1 hour 13 minutes	0,401
1 hour 14 minutes	0,401
1 hour 15 minutes	0,407
1 hour 16 minutes	0,411
1 hour 17 minutes	0,412
1 hour 18 minutes	0,416
1 hour 19 minutes	0,418
1 hour 20 minutes	0,420

Wavelength determination was determined from the middle standard solution of 6 mg/L with an operating time of 1 hour 14 minutes using 1,10 phenanthroline complex. Determination of the maximum wavelength was carried out in the wavelength range of 400-800 nm. This range was chosen because the 1,10 phenanthroline complex produces an orange-red color that shows maximum absorption at that wavelength. The 1,10 phenanthroline complex was used as a complexor because phenanthroline forms complexes over a long period of time (Morti *et al*., 2018) The results showed that the maximum length was located at 509 nm, with an absorbance of 2.097 nm (table 2).

Table 2. Operating time result data

Wavelength (nm)	Absorbance
509,39	2,097
442,10	1,618
424,70	1,507
354,90	1,727
345,30	1,929
342,00	2,031
335,70	2,251
326,80	2,803
321,00	4,000
318,30	4,000
315,40	4,000

An analytical method has good linearity if its calibration curve equation shows a correlation coefficient (r) value of more than 0.995 and a coefficient of determination (R^2) of more than 0.990. In this study, the r value obtained was 0.9986. The value of r which is close to 1 indicates that the data forms a perfect straight line, this proves that the curve between concentration and absorbance has a strong correlation. [7]. The regression equation obtained is y=0.1279x + 0.0809, with the slope value of the regression line is 0.1279 and the intercept value obtained is 0.0809 (Figure 1).

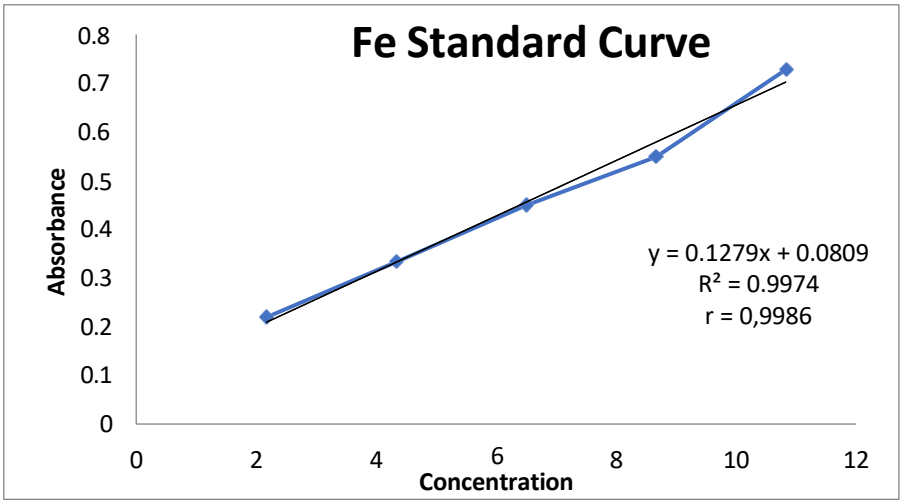

Figure 1. Fe Standard Curve

The detection limit and quantitation limit were determined statistically through linear line analysis generated from the standard curve. The results obtained showed that the detection limit value was 0.126 mg/L which is the lowest concentration of analyte in the sample that can be detected by the device. The limit of quantitation of 0.422 mg/L is the minimum concentration of analyte in the sample whose level can be measured accurately (table 3).

Table 3. LoD and LoQ results

Concentration (x) ppm	Absorbance (y)	y'	y-y'	(y-y')2
1	0,128	0,134026	-0,00603	3,63151E-05
2	0,196	0,212353	-0,01635	0,000267425
3	0,279	0,290679	-0,01168	0,000136407
4	0,345	0,369006	-0,02401	0,000576301
5	0,415	0,447333	-0,03233	0,001045435
			Total	0,002061883
			SD	0,0262163
			LoD	0,126675
			LoQ	0,422252

The %RSD results for wet-deconstructed blood tablet samples show that sample N has the highest %RSD value of 0.17% and the smallest %RSD value is in sample FF with a result of 0.03%. The average %RSD obtained in the measurement of blood tablet samples in wet deconstruction shows the result of 0.09%. Based on the %RSD value on the sample of blood tablets that are prepared by wet deconstruction, it shows a very thorough level of accuracy. The measurement results of %RSD on blood tablet samples prepared by dry deconstruction show that the FF sample has the smallest %RSD value of 0.070%. The largest %RSD value is in sample N with a result of 0.43%. The average %RSD generated in the measurement of blood tablet samples by dry deconstruction is 0.189%. The results of %RSD on blood tablet samples that are prepared by dry deconstruction show a very thorough level of accuracy because it has a %RSD value of less than 1% (table 4).

Table 4. Percentage of relative standard deviation

Sample	% RSD Dry Destruction	% RSD Wet Destruction	Terms
FF	0,070	0,03	
M	0,130	0,07	
S	0,17	0,09	<1%
L	0,12	0,08	
N	0,43	0,17	
Average	0.189	0.09	

The %recovery results for blood tablet samples prepared by wet deconstruction that the blood tablet sample M has %recovery with the highest value of 104.89% and sample N has the lowest %recovery results with a value of

82.24%. The average %recovery results obtained for blood supplement tablet samples prepared by wet deconstruction showed 96.70%.

The %recovery results for blood tablet samples that were prepared by dry deconstruction that the M blood tablet samples obtained %recovery with the highest percentage value of 107.58% and the N and FF blood tablet samples showed the lowest %recovery results with the value obtained 82.24%. The average %recovery obtained for blood tablet samples prepared by dry deconstruction showed a result of 95.11%. Analysis of Fe levels in blood tablet samples by dry deconstruction and wet deconstruction obtained accurate results because it has a percentage of recovery that falls into the required 80-120% range. Analysis of Fe (II) in blood tablet samples using the UV-Vis spectrophotometer method by dry deconstruction and wet deconstruction has met the method validation parameters including linearity, LoD, LoQ, precision so that accuracy is proven to be accurate (table 5).

Table 5. Percentage of recovery

Sample	Concentration mg/L	Dry Destruction	Mean ± SD	Wet Destruction	Mean ± SD
FF	10	80,88%	82,24 ± 1,90	97,10%	91,62± 4,75
	11	84,41%		88,91%	
	12	81,41%		88,83%	
M	5	105,77%	107,58 ± 1,62	100,50%	104,89 ± 8,83
	6	108,003%		99,08%	
	7	108,93%		115,24%	
S	1,5	103,28%	101,39 ± 2,44	96,16%	102,63 ± 6,46
	2,3	102,28%		102,74%	
	3,1	98,62%		109,02%	
L	2,5	106,46%	102,12 ± 3,78	91,19%	102,12 ± 3,78
	3,8	100,47%		96,34%	
	5,8	99,52%		101,77%	
N	0,9	80,80%	82,24 ± 1,90	92,59%	82,24 ± 1,90
	1,3	94,17%		87,08%	
	1,8	100,28%		83,32%	
Average			95.11		96,70

Comparison of analytical method validation results aims to ensure that the methods used produce accurate, precise data. By comparing the validation results, it can ensure that the method complies with accepted standards and can show better results from both methods. The results of the calculation of validation parameters for both methods, namely the dry deconstruction method and the wet deconstruction method can be seen in (table 6). Comparison of the results of the validation of precision analysis methods (%RSD) on blood supplement tablet samples prepared by wet deconstruction showed a result of 0.09 and dry deconstruction obtained a result of 0.189. Based on the results of the comparison of the validation of the precision test, it can be said that the blood supplement tablet samples prepared by wet deconstruction show a higher level of similarity compared to the dry deconstruction method. The smaller the %RSD value, the lower the level of variation between the results and the value is close to the average value. The %recovery value of the wet deconstruction method is 96.70% and the dry deconstruction method is 95.11%. This shows that the wet deconstruction method has a higher level of recovery or accuracy, namely the %recovery value is close to 100%, where the wet deconstruction method is able to produce results that are more accurate and closer to the actual value than the dry deconstruction method (Pratiwi & Sunarto, 2018). This shows that the wet desturksi method has a higher %recovery than dry deconstruction in providing the results of the recovery.

Table 6. Comparison of Analysis Method Validation Results

Method Validation	Dry Destruction	Wet Destruction	Acceptance Criteria
Linearity	0,9986		0,75 - 1
Detection Limit	0.126 mg/L		-
Quantitation Limit	0.422 mg/L		-
Precision (%RSD)	0,189%	0,09%	>1%

Method Validation	Dry Destruction	Wet Destruction	Acceptance Criteria
Accuracy (% recovery)	95,11%	96,70%	80%-120%

The results of the determination of Fe (II) levels in blood tablet samples show that samples prepared by wet deconstruction and dry deconstruction methods have levels above the detection limit and quantitation limit of the UV-Vis spectrophotometer so that the calculation of Fe (II) levels in the blood tablet samples can be said to be accurate. The levels of each sample of blood tablets added FF, M, S, L and N with wet deconstruction are 12,033 mg/L; 20,920 mg/L; 14,1388 mg/L; 5,0542 mg/L; 13,236 mg/L. The percentage of levels produced by blood supplement tablet samples with wet deconstruction is 91.52%; 100.09%; 96.15%; 100.96%; 100.67% (table 7). The percentage levels meet the requirements of Fe levels in the Indonesian Pharmacopoeia Edition II with a range of 90-110%.

Table 7. Determination of Fe (II) levels in blood supplement tablet samples

Sample	Wet Destruction		Kerning Destruction	
	Level (mg/L) ±SD	% Content ±SD	Level (mg/L) ±SD	% Content ±SD
FF	12.033±0,006	91.52±0,05	12.2786±0,004	93.39±0,03
M	20.920±0,008	100.09±0,04	3.6518±0,004	108.29±0,14
S	14.1388±0,006	96.15±0,03	14.627±0,003	99.47±0,02
L	5.0542±0,004	100.96±0,08	4.678±0,004	93.47±0,08
N	13.236±0,006	100.67±0,04	12.171±0,005	92.57±0,03

The results of levels for blood supplement tablet samples prepared by dry deconstruction showed the results of 12.27 mg/L; 3.65 mg/L; 14.627 mg/L; 4.67 mg/L and 12.171 mg/L. The percentage levels obtained by dry-deconstructed blood supplement tablet samples obtained results of 93.39%; 108.29%; 99.47%; 93.47%; and 92.57% (table 7). The percentage levels meet the requirements of Fe levels in the Indonesian Pharmacopoeia Edition II with a range of 90-110%.

CONCLUSION

The UV-Vis Spectrophotometric method has been validated as an appropriate method for analysis in blood supplement tablet samples prepared by dry deconstruction and wet deconstruction based on linearity, detection limit, quantitation limit, precision, and accuracy parameters. Linearity is shown with R2 of 0.9986; limit of detection of 1.10 mg/L; limit of quantitation of 3.62 mg/L; accuracy of dry deconstruction and wet deconstruction between 80-120%; and precision of dry deconstruction and wet deconstruction indicated by the percentage of RSD below 2%.

The results of quantitative testing showed the percentage content of blood supplement tablets samples of wet deconstruction and dry deconstruction of FF, M, S, L and N were 91.52%; 100.09%; 96.15%; 100.96%; 100.67% respectively. and 93.39%; 108.29%; 99.47%; 93.47%; and 92.57%.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

We declare that we have no conflict of interest

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