

# Determination of Isoniazid by Fe(II)-2,2'-Bipyridine Spectrophotometry

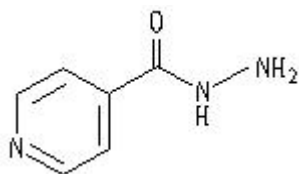
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**Abstract.** In acidic media, Fe(III) could be reduced to Fe(II) by isoniazid, Fe(II) reacts with 2,2'-bipyridine to form an orange complex with a maximum absorption wavelength of 522 nm. According to this principle, the content of isoniazid can be measured indirectly by measuring the amount of Fe(II). A new method for the determination of isoniazid by Fe(II)-2,2'-bipyridine spectrophotometry has been established. At the optimum conditions, the linear relationship is good between the mass concentration of isoniazid and absorbance of Fe(II)-2,2'-bipyridine complex in the range of 0.0004000-0.002800 mg/mL. The linear regression equation is  $A=0.0251+138.04C(\text{mg/mL})$ , and the linear correlation coefficient is 0.9995. This method is used to determine the content of isoniazid in isoniazid tablets, and the results are similar to those obtained by pharmacopoeia method.

## 1 Introduction

Isoniazid (the structure is shown in Figure 1) is mainly as one of the preferred drugs for effective treatment of various tuberculosis in clinical practice. But it has drug resistance, and can cause liver damage during the treatment process. Therefore, it is of great importance and significance for the determination of isoniazid content. So far, spectrophotometry[1-2], fluorescence spectrum method[3], flow-injection chemiluminescence analysis[4], capillary electrophoresis analysis[5-6], GC[7], HPLC[8-9], electrochemical method[10-11], and so on have been applied for the determination of isoniazid.



**Fig. 1.** The molecular structure of isoniazid

A new method for the determination of isoniazid by Fe(II)-2,2'-bipyridine spectrophotometry has been established. The various factors affecting the determination of isoniazid content are investigated. The results show that in acidic media, Fe(III) could be reduced to Fe(II) by isoniazid, Fe(II) reacts with 2,2'-bipyridine to form an orange complex with a maximum absorption wavelength of 522 nm. At the optimum conditions, the linear relationship is good between the mass concentration of isoniazid and absorbance of Fe(II)-2,2'-bipyridine complex, the content of isoniazid can be measured indirectly by measuring the amount of Fe(II). In the range of 0.0004000-0.002800 mg/mL, the

linear regression equation is  $A=0.0251+138.04C(\text{mg/mL})$ , and the linear correlation coefficient is 0.9995. This method is used to determine the content of isoniazid in isoniazid tablets, and the results are satisfactory.

## 2 Experimental

### 2.1 Equipment and reagents

723S spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd ) is used for photometric measurements. UV-2401 UV-visible spectrophotometer (The Shimadzu Corporation, Japan) is used for scanning the absorption spectrum.

Isoniazid standard solution: 0.1000 mg/mL. Fe<sup>3+</sup> solution: 0.1000 mg/mL, is prepared by NH<sub>4</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O and appropriate amount of 3.0 mol/L H<sub>2</sub>SO<sub>4</sub> solution. 2,2'-bipyridine (BPY) solution: 0.01998 mol/L. Buffer solutions of different pH was prepared as references 12.

All reagents are analytical reagent. Bidistilled water is used throughout.

### 2.2 Method

A given volume of 0.1000 mg/mL isoniazid standard solution or sample solution, 0.2000 mg/mL Fe<sup>3+</sup> solution 1.40 mL are added into a 25 mL ground color comparison tube, added water to 10.00 mL and mixed well. Aftering this mixture reacted for 30 min at 80°C in water bath and cooled back to room temperature, 0.01998 mol/L 2,2'-bipyridine solution 0.30 mL and

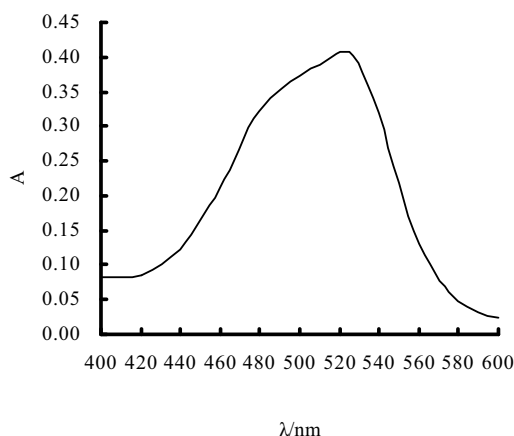
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pH=5.0 buffer solution 4.00 mL are added. The solution is diluted to 25.00 mL and mixed well, then, the absorbance is measured at 522 nm against the reagent blank after placing 10 min.

### 3 Results and discussion

#### 3.1 Maximum absorption wavelength

According to the experimental method, the absorption spectrum(400-600 nm) of Fe(II)-2,2'-bipyridine orange complex is shown in Figure 2. The maximum absorption wavelength of Fe(II)-2,2'-bipyridine orange complex is at 522 nm. So, the absorption wavelength 522 nm is selected.



**Figure 2.** Absorption spectrum

Isoniazid solution:0.30 mL; Fe<sup>3+</sup> solution:1.40 mL; BPY solution:0.30 mL; pH 5.0 buffer solution:5.00 mL; reaction temperature:80°C; reaction time:20 min; placing time:15 min.

#### 3.2 Reaction temperature

The effect of reaction temperature on absorbance is seen in table 1. It can be found that the absorbance of solution reaches greatest and almost constant when the reaction temperature is 70~100°C. Hence, 80°C is used.

**Table 1** Relationship between the reaction temperature and the absorbance

Temperature /°C	30	40	50	60
Absorbance	0.126	0.138	0.143	0.147
Temperature /°C	70	80	90	100
Absorbance	0.158	0.159	0.158	0.160

#### 3.3 Reaction time and placement time

The relationship between the reaction time and the absorbance can be seen in table 2. It is found that the absorbance of solution reaches greatest and keeps constant when the reaction time is 30 ~ 60 min. Therefore, 30 min has been chosen.

**Table 2** Relationship between the reaction time and the absorbance

Time/min	5	10	15	20
Absorbance	0.159	0.158	0.159	0.161
TimeT/min	30	40	50	60
Absorbance	0.168	0.166	0.166	0.165

When the placement time is 5~120 min, the experimental results of placement time on absorbance show that the absorption is not affected by the placement time. Thus, the placement time is selected for 10 min.

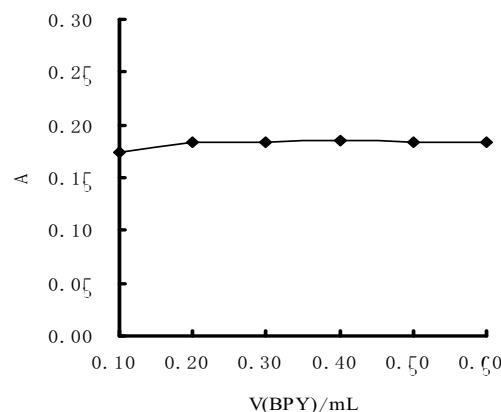
#### 3.4 pH buffer solution and its dosage

The effects of pH buffer solution on absorbance are considered. The results show that the absorbance of solution are maximal and remain almost constant when the pH is 3.6 ~ 5.6. Consequently, pH 5.0 buffer solutions is used.

The experimental results of the dosage of pH 5.0 buffer solution on absorbance showed that the absorbance reaches its maximum value and remains constant when the dosage of pH5.0 buffer solution is 3.00~8.00 mL. Therefore, the optimum dosage of pH buffer solution is 4.00 mL.

#### 3.5 The dosage of 2,2'-bipyridine

The effect of the dosage of 2,2'-bipyridine can be seen in Figure 3. From Figure 3, we can see that the absorbance reaches its maximum value and keep basically unchanged when the dosage of 2,2'-bipyridyl is 0.20~0.60 mL. So, 0.30 mL is identified as the optimum dosage of 2,2'-bipyridine.



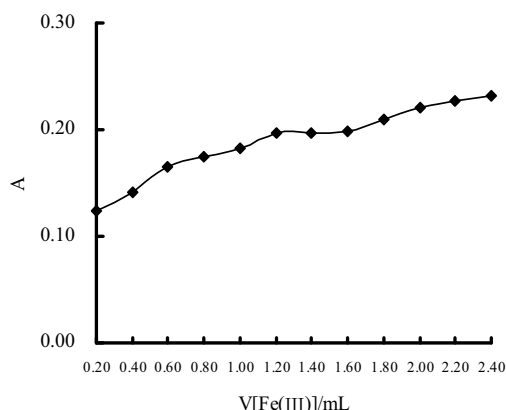
**Figure 3.** Effect of the dosage of 2,2'-bipyridine

Isoniazid solution:0.30 mL; Fe<sup>3+</sup> solution:1.40 mL; pH 5.0 buffer solution:4.00 mL; reaction temperature:80°C; reaction time:30 min; placing time:10 min.

#### 3.6 The dosage of Fe(III)

The relationship between the the dosage of Fe(III) and the absorbance is showed in Figure 4. It is found that the

absorbance reaches greater and remains constant when the dosage of Fe(III) is 1.20 mL~1.60 mL. So, 1.40 mL of Fe(III) is employed.

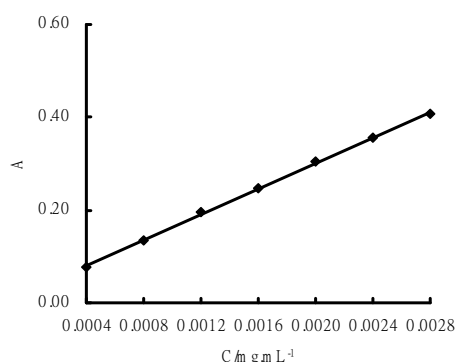


**Figure 4.** Effect of the dosage of Fe(III)

Isoniazid solution:0.30 mL; BPY solution:0.30 mL; pH 5.0 buffer solution:4.00 mL; reaction temperature:80°C; reaction time:30 min; placing time:10 min.

### 3.7 Standard curve

According to the experimental method, under the selected conditions (0.2000mg/mL Fe<sup>3+</sup> is 1.40 mL, 0.01998mol/L 2,2'- bipyridine is 0.30 mL, pH 5.0 buffer solutions is 4.00mL, reaction temperature is 80°C, reaction time is 30min, placement time is 10 min), the different concentration of isoniazid standard solutions are prepared and the absorbances of these solutions are measured at 522 nm against the reagent blank. Then draw with concentration as horizontal coordinate and absorbance as vertical coordinate, the standard curve is showed in Figure 5. In the range of 0.0004000-0.002800 mg/mL, the Beer's law is obeyed between the concentration of isoniazid and the absorbance, the linear regression equation is  $A = 0.0251 + 138.04C$  (mg/mL) and the correlation coefficient is 0.9995.



**Figure 5.** Standard curve

Fe<sup>3+</sup> solution:1.40 mL; BPY solution:0.30 mL; pH 5.0 buffer solution:4.00 mL; reaction temperature:80°C; reaction time:30 min; placing time:10 min.

### 3.8 Sample analysis

20 tablets of isoniazid tablet are weighed 2.4039 g, 0.5098 g powder of isoniazid tablet is weighed precisely after grinding and biending. The powder of isoniazid tablet is dissolved in bidistilled water and is transferred into a 250 mL volumetric flask, the solution is diluted to 250.0 mL, mixed well and filtered. 10.00mL filter liquid is transferred into a 200 mL volumetric flask, then this filter liquid is diluted to 200.0 mL with bidistilled water, mixed well. This is the isoniazid sample solution, and the solution is preserved at 4°C, shielding from light.

When isoniazid sample solution(0.30 mL), 0.2000 mg/mL Fe<sup>3+</sup> solution(1.40 mL), 0.01998 mol/L 2,2'-bipyridine solution(0.30 mL) and pH=5.0 buffer solution (4.00 mL) are added. Based on the experimental method, the content of the isoniazid in isoniazid tablet is determined by controlling the reaction temperature is 80°C, reaction time is 30 min and placing time is 10 min. Meanwhile, the recovery tests of standard addition are performed and the content of isoniazid in isoniazid tablet is determined by pharmacopoeia method. The results as show in Table 3.

**Table 3** The content of isoniazid in isoniazid tablets n=5

Sample	Isoniazid tablet
Proposed method(mg·tablet <sup>-1</sup> )	100.7
RSD (%)	1.3
Pharmacopoeia method[13] (mg·tablet <sup>-1</sup> )	96.1
Added (µg·mL <sup>-1</sup> )	0.4000 0.8000
Recovered(µg·mL <sup>-1</sup> )	0.3997 0.7724
Recovery(%)	99.9 96.6

It is seen from table 3 that the content of isoniazid in isoniazid tablet is 100.7 mg·tablet<sup>-1</sup> by this proposed method, and the content of isoniazid in isoniazid tablet is 96.1 mg·tablet<sup>-1</sup> by pharmacopoeia method. It shows that the two results are similar. It is indicates that the content of isoniazid in isoniazid tablet can be accurately determined by Fe(II)-2,2'-bipyridine spectrophotometry.

### 4 Conclusion

In this paper, a new method for the determination of isoniazid by Fe(II)-2,2'-bipyridine spectrophotometry is reported. This method has been successfully used for the determination of isoniazid in isoniazid tablets, and the results are similar to the results of pharmacopoeia method. It is clearly obvious that the determination of isoniazid by Fe(II)-2,2'-bipyridine spectrophotometry has certain practical significance and application prospect.

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