UV SPECTROPHOTOMETRIC ABSORPTION METHOD FOR THE DETERMINATION OF ISONIAZID IN ACTIVE PHARMACEUTICAL INGREDIENT

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Abstract:

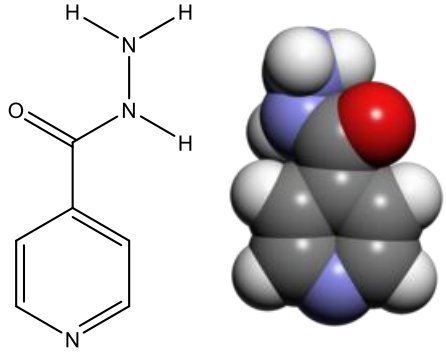
Simple, rapid, sensitive, precise and specific UV spectrophotometric method for the determination of isoniazid (INH) in active pharmaceutical ingredient were developed and validated. A simple double beam UV spectrophotometric method has been studied with different parameters such as linearity, precision, repeatability, accuracy as per ICH guidelines. Measurement of absorption at maximum wavelength in water as reference isoniazid were found at 262 nm. The drug obeyed the Lambert - Beer's law in concentration range 10-40 µg/mL with correlation coefficient was 0.9999. The proposed method is precise, accurate and reproducible and can be used for routine analysis of isoniazid in active pharmaceutical ingredient.

Keywords: *INH; Isoniazid; UV spectrophotometry.*

1. Introduction

According to Chakraborty S., Rhee K.Y. (2015), "*Tuberculosis Drug Development: History and Evolution of the Mechanism-Based Paradigm. Cold Spring Harb Perspect*", isoniazid was first made in 1952. Isoniazid, also known as pyridine-4-carbohydrazide, or isonicotinylhydrazide (INH), is a bactericidal agent active against organisms of the genus *Mycobacterium*, specifically *M. tuberculosis*, *M. bovis* and *M. kansasii*. It is a highly specific agent, ineffective against other microorganisms. Isoniazid is bactericidal when mycobacteria grow rapidly, and isoniazid is bacteriostatic when mycobacteria grow slowly. For active tuberculosis, it is often used together with rifampicin, pyrazinamide, and either streptomycin or ethambutol.

Figure 1. Chemical structures of isoniazid



2. Research overview

Quantitative analysis of isoniazid can be performed by non-aqueous acid-base titration (Swamy N., Kanakapura B., and Vinay K. (2015), *"Titrimetric assay of isoniazid with perchloric acid in non-aqueous medium"*), and bromate titration (*Vietnamese pharmacopoeia V*). These are all chemical methods that often encounter many errors. Besides, non-aqueous acid-base titration using concentrated chemicals can cause environmental pollution and affect health due to evaporation.

According to Kalia S.B., Kaushal G., and Verma B.C. (2006), "*Spectrophotometric method for the determination of isoniazid",* carbon disulphide transforms isoniazid in methanol medium into isonicotinyldithiocarbazic acid. The latter reacts with uranyl acetate in the same medium rapidly and quantitatively to form soluble bright yellow uranyl isonicotinyldithiocarbazate complex, has been made the basis of a simple, rapid and sensitive spectrophotometric method for the determination of isoniazid. The wavelength was measured at 410 nm. According to Oga E. (2010), "*Spectrophotometric Determination of Isoniazid in Pure and Pharmaceutical Formulations using Vanillin"*, the method was based on the coupling of isoniazid and vanillin in an ethanolic hydrochloric acid with acidic medium and the spectrophotometric was determined at the absorption maximum (405 nm). In the study of Gowda B.G. *et al.* (2002), *Spectrophotometric determination of isoniazid in pure and pharmaceutical formulations,* a rapid and sensitive spectrophotometric method was presented for the determination of INH in pure base on the oxidation of 4,5-dihydroxy-1,3-benzenedisulfonic acid by sodium metaperiodate followed by oxidative coupling with INH in an alkaline medium. These physicochemical methods all have high accuracy but use complicated solvent or need reaction intermediates.

This article presents the research on developing a method to quantify the content of isoniazid using ultraviolet absorption spectroscopy. This physicochemical method is more accurate than the chemical method and uses an environmentally friendly and inexpensive distilled water solvent.

3. Research methods

3.1. Instruments

Shimadzu UV-1800 double beam spectrophotometer was used to record the spectra of sample and reference solutions using a pair of quartz cells of 10mm path length. All weighing was carried out on Shimadzu AUX220 analytical balance.

3.2. Materials

Standard sample of isoniazid with a purity of 99% were provided from National Institute of Drug Quality Control. Isoniazid in active pharmaceutical ingredient were procured from local chemical store and analytical grade water (Purity Analytical).

3.3. Data Collection Methods

MS Excel software with Data Analysis was used to statistically analyze data-including mean value, standard deviation (SD), relative standard deviation (RSD).

3.4. Methods

3.4.1. Determination of λ_{max}

50 mg of isoniazid was dissolved exactly in 50 mL of analytical grade water. After that, 500 µL of the solution was added in 25 mL of analytical grade water using a 25 mL volumetric flask. The final solution was subjected to scanning from 200-400 nm and maximum absorption was determined.

3.4.2. Quantitative methods

50 mg of isoniazid was dissolved exactly in 50 mL of distilled water, then 500 µL of the solution was dissolved in 25 mL of distilled water.

The process was carried out in parallel to make a standard sample with standard isoniazid. The optical density values the two solutions were determined at λ_{max} wavelength using a pair of quartz cells of 10mm path length. The blank sample was distilled water.

3.5. Method of validation

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996 *(https://www.ich.org/.)*

3.5.1. Linearity

The linearity of the proposed assay was studied in the concentration range 10 - 40 µg/mL at 262 nm. The calibration data showed a linear relationship between concentrations.

3.5.2. Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration. A known amount of active drug was added to each sample solution and dissolved in 100 mL of the volumetric flask with analytical grade water and measuring the absorbance at 262 nm.

3.5.3. Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the % RSD was calculated.

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

LOD is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under stated experimental conditions. The LOD and LOQ for INH were determined according to the ICH guideline.

$$LOD = 3.3\sigma / S$$

$$LOQ = 10\sigma / S$$

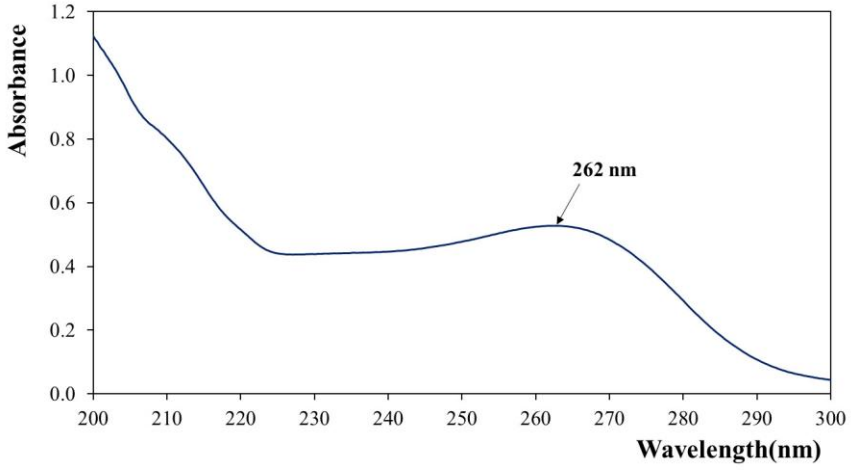
Where, σ = Standard deviation of the y-intercept of calibration curves, S = Slope of the calibration curve.

4. Research results

4.1. Determination of λ_{max}

The wavelength at 262 nm was attributed to the maximum absorption of INH. The UV spectroscopy of the drug is presented in Fig. 2.

Fig. 2. The UV spectroscopy of INH



4.2. Quantitative methods

50mg of isoniazid ingredients was diluted with enough 50mL of distilled water. Then, 500µL of the mixed solution was further diluted with enough 25mL of distilled water.

Proceed in parallel to make a standard sample with standard isoniazid. The optical density of the two solutions was measured at wavelength λ_{max}, using a pair of quartz cells of 10mm path length. The blank sample is distilled water.

The percentage of isonioazid is calculated according to the formula:

$$C_{th} = \frac{A_t.m_c}{A_c.m_t} . C_c$$

In there:

C_{th}: % isoniazid content in the test sample.

C_c: % isoniazid content in the standard sample.

A_t: Absorbance of the test solution.

A_c: Absorbance of standard solution.

m_t: Mass of isoniazid powder test.

m_c: Mass of standard isoniazid powder.

4.3. Method of validation

4.3.1. Linearity

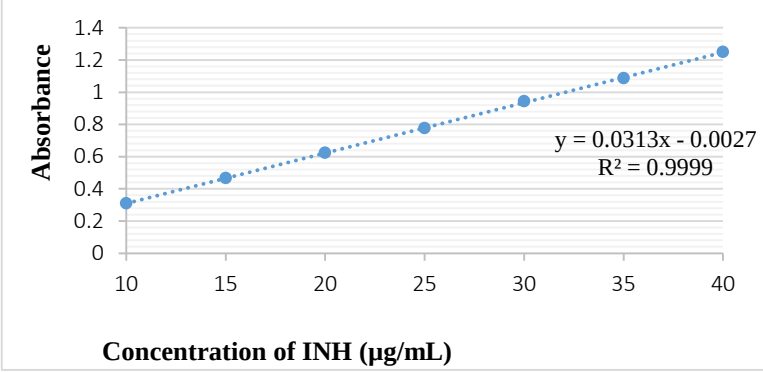
The linearity of method was determined at concentration level ranging from 10 to 40 µg/mL. The correlation coefficient value was found to be (R^2) 0.9999.

Results are shown in Table 1 and Fig.3.

Table 1. Linearity studies

Concentration of INH (µg/mL)	Absorbance
10	0.310
15	0.466
20	0.623
25	0.777
30	0.943
35	1.087
40	1.249

Fig. 3: Isoniazid standard curve



4.3.2. Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table 2. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

Table 2. Accuracy studies

Accuracy (%)	Quantity weighed (mg)	Quantity found (mg)	Recovery (98-102%)
80	40.300	40.572	100.67
100	50.100	50.251	100.30
120	60.200	60.647	100.74

4.3.3. Precision

The % RSD for different sample of precision was found to be 0.012 and it is within cceptance criteria represented in Table 3.

Table 3. Precision studies

Sample	Absorbance	Percentage (%)	
1	0.635	103.52	
2	0.639	104.38	
3	0.614	100.49	Mean value = 102.09%
4	0.627	102.21	
5	0.621	101.03	SD = 1.228 %
6	0.626	102.05	
7	0.624	101.72	RSD = 1.2%
8	0.623	101.76	
9	0.633	102.99	

Sample	Absorbance	Percentage (%)	
10	0.621	100.83	

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

The LOD and LOQ values computed from the stated formulae were found to be 1.29 µg/mL and 3.92 µg/mL for INH.

5. Discussions

Research results showed that the isoniazid quantification method using ultraviolet absorption spectrophotometry achieves linearity, accuracy and precision according to the validation requirements of ICH guidelines.

These advantages of the method include the following: Instrumental methods produce more accurate results than traditional methods. Water solvent is environmentally friendly. And UV-VIS spectrophotometer is not too expensive, and is commonly used.

These disadvantages of the method include the following: Ultraviolet absorption spectrophotometry method need standard substances. Standard substances are often expensive.

6. Conclusion

A method for the estimation of isoniazid in pure form has been developed. From the spectrum of isoniazid, it was found that the maximum absorbance was 262 nm in analytical grade water. A good linear relationship was observed in the concentration range of 10-40 µg/mL. The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate and precise for the estimation of isoniazid in pure. Hence, the method could be considered for the determination of isoniazid in quality control laboratories.

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PHƯƠNG PHÁP ĐO QUANG PHỔ HẤP THỤ TỬ NGOẠI ĐỂ ĐỊNH LƯỢNG ISONIAZID TRONG NGUYÊN LIỆU LÀM THUỐC

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Tóm tắt:

Phương pháp đo quang phổ hấp thụ tử ngoại đơn giản, nhanh chóng, nhạy, chính xác và đặc hiệu để xác định isoniazid (INH) trong nguyên liệu làm thuốc đã được xây dựng và thẩm định. Một phương pháp đo quang phổ hấp thụ tử ngoại với 2 chùm tia đơn giản đã được xây dựng và thẩm định với các thông số khác nhau như độ tuyến tính, độ chính xác, độ lặp lại theo hướng dẫn của ICH. Phương pháp đo quang phổ UV-VIS, cực đại hấp thụ của dung dịch isoniazid trong nước được tìm thấy ở 262 nm. Thuốc tuân theo định luật Lambert - Beer và cho thấy mối tương quan tốt. Định luật Lambert - Beer được tuân theo trong khoảng nồng độ 10-40 µg/mL với hệ số tương quan là 0,9999. Phương pháp được đề xuất là chính xác, có độ lặp lại và có thể được sử dụng để phân tích thường quy isoniazid trong nguyên liệu làm thuốc.

Từ khóa: *INH; Isoniazid; Quang phổ hấp thụ UV.*