According to the best of our knowledge, there is no published method for finding the zero crossing value \[17\].

Simultaneous estimation is a simple, accurate, and precise method with no interference from excipients. It is not required long time to sample preparation, no many solvents, no complicated mathematical equations for treatment and prevent the emergence of resistance, one of the principles used is to prioritize the drug combinations \[3\]. One such combination is a INH and pyridoxine hydrochloride (PDX) \[3\].

**ABSTRACT**

**Objective:** This study aimed to develop spectrophotometry ultraviolet (UV) method by area under curve (AUC) to determine the levels of isoniazid (INH) and pyridoxine hydrochloride (PDX) in commercial tablets.

**Methods:** Spectrophotometry UV method by AUC tested is carried out by optimizing the type of solvent, methanol, mixture of methanol, and phosphate buffer pH 4, pH 5, and pH 6 at a ratio of 90:10. The AUC value of each spectrum in various concentrations is calculated to selected wavelength range analysis. Then, this method was applied to determine the levels of INH and PDX in commercial tablets.

**Results:** The application of AUC spectrophotometry UV method for determining the levels of INH and PDX was performed on wavelength range 262.2–272.2 nm and 289.8–299.8 nm, respectively. The selection of wavelengths based on wavelengths gives the best result. The mean % recoveries were found to be at 99.69% and 101.09% for INH and PDX, respectively.

**Conclusion:** The method is successfully applied to analyze INH and PDX in pharmaceutical formulation simultaneously with no interference from excipients as indicated by all validation parameters which were within the acceptable range.

**Keywords:** Isoniazid, Pyridoxine hydrochloride, Area under curve, Spectrophotometry ultraviolet.

**INTRODUCTION**

Isoniazid (INH) was introduced in 1952 as a drug that is quite effective in the treatment of tuberculosis \[1\]. Its chemical name is pyridine-4-carbohydrazide \[2\]. It was soluble in water, rather difficult to dissolve in ethanol, difficult to dissolve in chloroform and ether. Based on the germicidal properties of its tuberculosis, to obtain the effectiveness of treatment and prevent the emergence of resistance, one of the principles used is to prioritize the drug combinations \[3\]. One such combination is a INH and pyridoxine hydrochloride (PDX) \[3\].

The chemical name of PDX is 5-hydroxy-6-methyl-3-pyridine-dimethanol hydrochloride \[2\]. It is also called as Vitamin B6 \[2\]. It was freely soluble in water, soluble in methanol, and not soluble in ether \[1\]. It was given to the patients taking INH to prevent peripheral neuropathy and central nervous system effects that are associated with the use of INH \[3\].

Some analytical methods have been developed for the quantitative analysis of INH or PDX in pure individual drugs, in their combination, in the pharmaceutical dosage forms, and in the biological fluids. These methods are Fourier transform infrared \[4\], voltammetry assay \[5,6\], spectrophotometry ultraviolet (UV) \[7-15\], polarography \[12\] and chemiluminescence \[13\], reverse phase (RP)-liquid chromatography (LC) \[14\], RP-high-performance LC \[15\], and high-performance thin LC \[16\].

Spectrophotometry UV with an area under curve (AUC) method for simultaneous estimation is a simple, accurate, and precise with no interference from excipients. It is not required long time to sample preparation, no many solvents, no complicated mathematical equations and no derivatization processes for finding the zero crossing value \[17\]. According to the best of our knowledge, there is no published spectrophotometry UV with AUC method for simultaneous estimation INH and PDX in their combination.

**METHODS**

**Reagents and chemicals**

All material and reagents were analytical reagent grade. The raw standard of INH was purchased from Zhejiang Jiangbei Pharmaceutical Industry, and the raw standard PDX was purchased from Jiangxi Tianxin Pharmaceutical Industry. Commercially available tablets Pehadoxin Forte® (containing 400 mg INH and 10 mg PDX, Phapros, Indonesia) and tablets TB Vit 6® (containing 400 mg INH and 25 mg PDX, Meprofarm, Indonesia) were obtained from a local pharmacy.

**Instruments and apparatus**

The spectrophotometric measurements were carried out on a Shimadzu-UV 1800 Spectrophotometer, matched quartz cells 1 cm, and UV probe 2.42 software was used for all spectral measurements. Benco analytical balance, Branson 1510 Ultrasonicator, and Hanna pH meter were also used.

**Optimizing of Solvent**

Optimizing of solvent is performed by measuring the absorbance of INH and Vitamin B6 in methanol, a mixture of methanol, and phosphate buffer pH 4, pH 5, and pH 6 in the ratio (90:10) at the wavelength range of 200–400 nm. It was observed that the solutions in methanol showed suitable conditions for the simultaneous estimation of both drugs. Methanol was selected as the solvent for further study.

**Preparation of standard solution**

Accurately weighed 50 mg of INH and PDX standard was separately transferred into 50 ml volumetric flask and dissolved in methanol to give solutions containing 1000 µg/ml INH and PDX.
Selection of analytical wavelength
The solutions of INH and PDX were prepared in diluent by appropriate dilution and the spectrum was recorded. The absorption spectra of the solutions prepared at different concentrations of INH (5–15 μg/ml) and PDX (6–18 μg/ml) were scanned in the range 200–400 nm. The AUC value of each spectrum in various concentrations is calculated to get the analytical wavelength.

Assay of tablet formulation by AUC spectrophotometry UV method
The content of twenty tablets was weighed accurately. A powder quantity equivalent to 69.1 mg INH and 3.21 mg PDX was accurately weighed and transferred to the volumetric flask of 50 ml. Then, 15 ml of methanol was transferred to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with diluent. The above solution was filtered through Whatman filter paper (0.45 μ). The solution was made up to the mark with diluent to give a solution containing 12 μg/ml of INH and 12 μg/ml of PDX (by addition PDX standard solution). The resulting solution was analyzed by the proposed method. The quantification analysis was carried out by keeping these values to the straight line equation of calibration curve.

Method validation
The method was validated based on linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). It was validated according to the International Conference on Harmonization guidelines.

RESULT AND DISCUSSION
Selection of analytical wavelength
Selection of analytical wavelength is carried out by calculating the AUC value of each spectrum in various concentrations at the wavelength range 255–275 nm for INH and the 281–295 nm wavelength range for PDX. The selected wavelength range is the best linear relation between its AUC value and its concentration that indicated by the correlation coefficient value (value r is ≤1) closed to one [18].

The selected wavelengths to determine INH and Vitamin B6 by spectrophotometry UV with AUC method were 262.2–272.2 nm and 289.8–299.8 nm, respectively (Fig. 1).

Based on Fig. 1, we can conclude that no overlapping AUC of each wavelength ranges both for INH (262.2–272.2 nm) and PDX (289.8–299.8 nm) and affecting one to another. According to this result, the spectrophotometry UV with AUC method can be used to simultaneously determine the drug content consisting of more than one component [19].

Method validation
The low values of percent relative standard deviation are indicative of the precision and reproducibility of the method. The resulting mixtures were assayed according to the above-stated procedure, and the results were calculated as the percentage of analyst recovered. LOD and LOQ values were indicated that the method shows high sensitivity. The good recovery values assure the high accuracy of the proposed method.

Validation parameters for this method are shown in Table 1.

| Parameters       | INH          | PDX          |
|------------------|--------------|--------------|
| Linearity        | 0.09979      | 0.9989       |
| Accuracy (%)     | 99.69        | 101.09       |
| Precision (RSD) (%) | 1.17       | 0.73         |
| LOD              | 0.0033       | 0.0312       |
| LOQ              | 0.0110       | 0.1040       |

PDX: Pyridoxine hydrochloride, INH: Isoniazid, RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

Table 1: Result of quantification of INH and PDX in commercial tablet

| Commercial tablet | Drugs content | Amount found (mg/tablet) | Label claim (mg/tablet) |
|-------------------|---------------|--------------------------|-------------------------|
| Tablet P          | INH           | 398.04±0.06              | 400                     |
|                   | PDX           | 9.945±0.59               | 10                      |
| Tablet T          | INH           | 397.48±0.18              | 400                     |
|                   | PDX           | 25.25±0.49               | 25                      |

PDX: Pyridoxine hydrochloride, INH: Isoniazid

The proposed method provides a simple, accurate, and precise quantitative analysis for the estimation of INH and PDX as a binary mixture. The proposed method is simple as there is no need for solvent extraction and direct as it estimates each component independent of the other and also low cost and harmless to the environment. Hence, it could be applied in the routine simultaneous analysis in quality control laboratories.

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CONFLICT OF INTEREST
We declare that there is no conflict of interest.
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