International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.09, pp 191-196, 2018

# Application of High Performance Thin Layer Chromatography-Densitometry and UV- Visible Spectrophotometry for the Simultaneous Determination of Thiamine in Green Beans

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**Abstract :** Two methods are described for the simultaneous determination of thiamine in green beans. The green beans were grinded and thiamine was extracted as bases into distilled water, separated by the first method, HPTLC silica gel 60 $F_{254}$ plate using methanol: water: acetic acid: ammonia (5:4.5:0.5:0.75) as mobile phase followed by densitometry measurement of its spot. The second method, a highly sensitive colour reaction has been developed. Thiamine was reacted with bromothymol blue to form an ion association complex in a weak base aqueous solution in the presence of some solubilization agents such as polyvinyl alcohol and analyzed by using UV- visible Spectrophotometry. The solution was measured at a maximum absorbance length of 430.5 nm. The first method showed that the detector response was linear for concentrations between 100-500 µg/ml (r=0.998). The limits of detection and quantitation were 33.7 µg/ml and 113.1 µg/ml, respectively. The second method was found to offer good linearity (18-26 µg/ml, r=0.998) with 0.6 µg/ml limit of detection and 1.9 µg/ml limit of quantitation. Thiamine contents from both methods were analyzed. The result showed that average contents of thiamine from both methods were 0.0396% and 1.0009%, respectively. The two proposed methods were successfully applied to the determination of thiamine in green beans.

**Keywords :** Green beans, HPTLC-Densitometry, Spectrophotometry UV/Vis, Thiamine.

## Introduction

Vitamin is an organic compound distinct from fats, carbohydrates, and proteins and a natural component of foods in which it is usually present in minute amounts for normal physiological function[3].Vitamin is classified based on their solubility in water or oil (fats). Vitamin C and B - complex are water soluble whereas Vitamin A, D, E and K are fat soluble dissolved in oil or in melted fat[11]. Thiamine (Vitamin $B_1$) is the trivial designation of a specific compound, 3-(4-amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)- 4-methylthiazolium, which is sometimes also called vitamin $B_1$ and widely distributed in foods



**DOI= http://dx.doi.org/10.20902/IJCTR.2018.110925**

such as green beans, but most contain only low concentrations of the vitamin.The greatest concentrations of thiamine in grains are typically found in the scutellum (the thin layer between the germ and the endosperm) and the germ[3].Thiaminehelps to prevent from heart disease[6],brain disorder[2], neurological problems[4], cures Beriberi[12], renal disease[9],and diabetic peripheral neuropathy[14].

Green bean is widely known as one source of thiamine and it is a popular bean in Indonesia, therefore the green bean is easily obtained and the price is relatively inexpensive. However, thiamine is unstable and easily degraded by heating and at high temperature, partially lost in cooking. monitoring thiamine loss during processing is important for optimal nutrient content in the final food products. Moreover, the content of thiamine in foods needs to be properly controlled to satisfy the guidelines set by the governmental authorities and need more analytical methods to analyze thiamine in dietary sources.

Several methods have been already reported forthe quantitative determination of thiamine in food and clinical analysis, includingspectrophotometry[1], high performance liquid chromatography (HPLC)[15], spectrofluorimetry[16], liquid chromatography–mass spectrometry (LC-MS)[7], and TLC-Densitometric[10].In all these methods, the most common methods used are high performance liquid chromatography and spectrophotometry.One of analytical methods that can be used for determining thiamine is High Performance Thin Layer Chromatography (HPTLC)-densitometry. HPTLC is an advanced form of instrumental TLC and it is generally used with an unmodified silica layer as stationary phase on precoated plates and slit-scanning densitometry with UV–vis light as the detection technique[13]. HPTLC has simplicity of procedure, efficiency with small amounts of sample, parallel analysis of samples, multiple nondestructive methods for detection, numerous options for developing solvents, low consumption of solvents, time and cost effectiveness (In fact, the expenses associated with providing solvents and maintenance are much lower in comparison with HPLC)[5]. These advantages enable this method to be an important alternative to other chromatographic techniques such as HPLC for determining thiamine in green beans. Another method used in this research is using triphenylmethane acid dyes method that was described in the previous research[8]. This method was modified by using bromothymol blue as a dye and reacted to thiamineto form an ion association complex in a weak base aqueous solution in the presence of some solubilization agents such as polyvinyl alcohol and analyzed by using UV-visible Spectrophotometry.In this research, the objective of the present work was to develop simple, rapid, and accurate methods to assay thiamine in green beansby High Performance Thin Layer Chromatography-Densitometry and UV- visible spectrophotometer and to compare the results by using these methods.

## Experimental

### Sample collection and preparation

Green beans (*Phaseolusradiates L.*)were purchased from local farmers inPadang West Sumatera Indonesia which were already in sterile packaging for trafficked. 100 grams of green beans were grinded before analysis. thegreen bean powders were sieved with a mesh width 500 μm and these sieved powders were used for the further analysis.

### Chemicals and Reagents

HPTLC analyses were performed on Merck 20 cm × 10 cm (0.25 mm) plates. Thiaminused as standard material was purchased from Indofarma Pharmaceutical Company. All the reagents used in the experiment were of analytical grade and were supplied by Merck, Darmstadt, Germany.

### Preparation of Sample

A weight of 5 grams of green bean powder wasaccurately weighed then it was taken in 50 mL Erlenmeyer beaker and subsequently distilled water was adjusted to the mark.the solution was shaken and filtered. 50 mL of filtrate was used for analysis.

**Preparation of Standard Solution**

A weight of 50 mg of standard thiamine was accurately weighed, quantitatively transferred into a 100 ml volumetric flask, dissolved in distilled water and the volume was adjusted with the same solvent.

**Chromatographic Procedure**

2 µL of sample and standard solutions were applied as bands of 4 mm wide, 0.3 mm high and 6 mm apart in the form of bands on pre-coated HPTLC silica gel plates 60 $F_{254}$(20 cm × 10 cm with 250 µmthickness) by means of band applicator CAMAG Linomat 5®sample applicator equipped with a 100 µL Hamilton® syringe. Samples were applied at 15 mm from the bottom edge of the chromatographic plate. The plates were allowed to dry for 15 minutes before elution using a solution of methanol/water/acetic acid/ammonia (5:4.5:0.5:0.75) as mobile phase. Thedeveloping distance was 85 mm, measured from the lower edge of the plates. The plates were allowed to dry for 15 minutes for visualization of the spots using CAMAG® UV Cabinet dual wavelength, 254/366 nm. Migration distances were measured and retention factors (RF) were calculated. Chromatograms were obtained by reading the plate using adensitometric scanning (CAMAG® TLC scanner 4 and the WinCATS© 4.3 software). Unknown samples extracts are spotted in triplicate.Evaluation was done by peak area measurement with linear regression.

**Spectrophotometric Procedure**

5 mL of sample was taken in a 25 ml calibrated flask. 1.5 mL of ammonia, 3 mL of 0.05% bromothymol blue and 1.0 mL of 1% polyvinyl alcohol were added to it. The volume was adjusted to the mark with distilled water. Then, 1.5 mL of this solution was taken and transferred to a further 10 mL volumetric flask. The volume was adjusted using distilled water and the absorbance was measured in a 1-cm cell at their own maximum absorption wavelength and maximum fading wavelength against the reagent blank using UV-visible Spectrophotometry (Shimadzu® UV-1800).Unknown samples extracts are determined in triplicate.Evaluation was done by absorbance measurement with linear regression.

**Calibration curve**

The calibration curves wereconstructed using standard solution of thiamine. The standard solution was applied to the plate corresponding to a concentration of 100-500 µg/mlfor HPTLC-densitometricmethod and18-36 µg/ml for spectrophotometric methodfollowed by spectrophotometric procedure as described for the preparation of a 5-point calibration curve.

## Result and Discussion

The thiamine contentsof green beans were evaluatedand quantified by using HPTLC-densitometric and UV-Vis spectrophotometric.In these studies, the methods were used by three replicate studies. The first used method was HPTLC-densitometry. HPTLC has recently been becoming more significant for quality control of thiamine. The availability, sensitivity and low cost are the advantages of this method. The optimum conditions for analysis thiamine by HPTLC was chosen experimentally by considering the effects of several factors such as the solution concentration, the ratio of solvents in the eluent and the type of HPTLC plates. In this study, Mixture of several solvents as mobile phase was tried to analysis of thiamine. The solvent system used was system methanol/water/acetic acid/ammonia (5:4.5:0.5:0.75) which gave good resolution and good chromatogram with $R_f$ value 0.50± 0.05. A satisfactory separation was obtained permitting simple and fast scanning of the components on the plates directly under UV-densitometer. The results showed linear relationship between the peak areas and the thiamine concentrations is shown in Figure 2. The plot was linear in the range of 100-500 µg/ml ($y = 5.815x + 290.7$, $r^2 = 0.998$). The five-point calibration was repeated many times and was also found to have a linear regression correlation coefficient of 0.998 which were showed accuracy of experiments. Limit of detection (LOD) and limit of quantification (LOQ) were33.7 and 113.1µg/mL, respectively.

The second method used was UV-Vis spectrophotometric. The wavelength of maximum absorption (λmax) of thiamine was measured and the wavelength of 430.5 nm was chosen for quantification of thiamine. The UV Spectra of thiamine is shown in Figure 1.

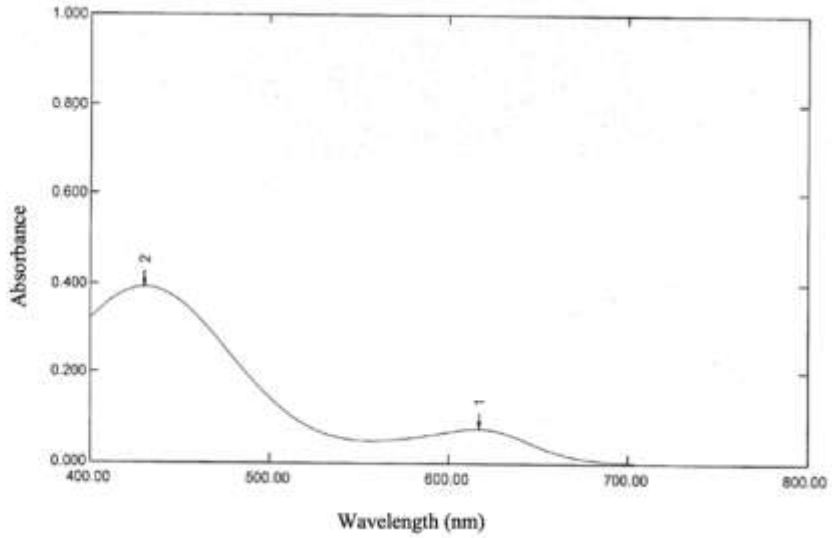


**Figure 1. UV spectrum of Thiamine**

The result showed linear relationship between absorbance and thiamine concentrations in range of 18-36 µg/mlwith regression equation y= 0.024x-0.104, $r^2$ = 0.998 as shown in Figure 2. Limit of detection (LOD) and limit of quantification (LOQ) for this study were 0.6 and 1.9 µg/mL, respectively. From these studies, it can be concluded that low limit of detection and limit of quantification were showed sensitivity of UV-Vis spectrophotometric method compared to the HPTLC-densitometric method.

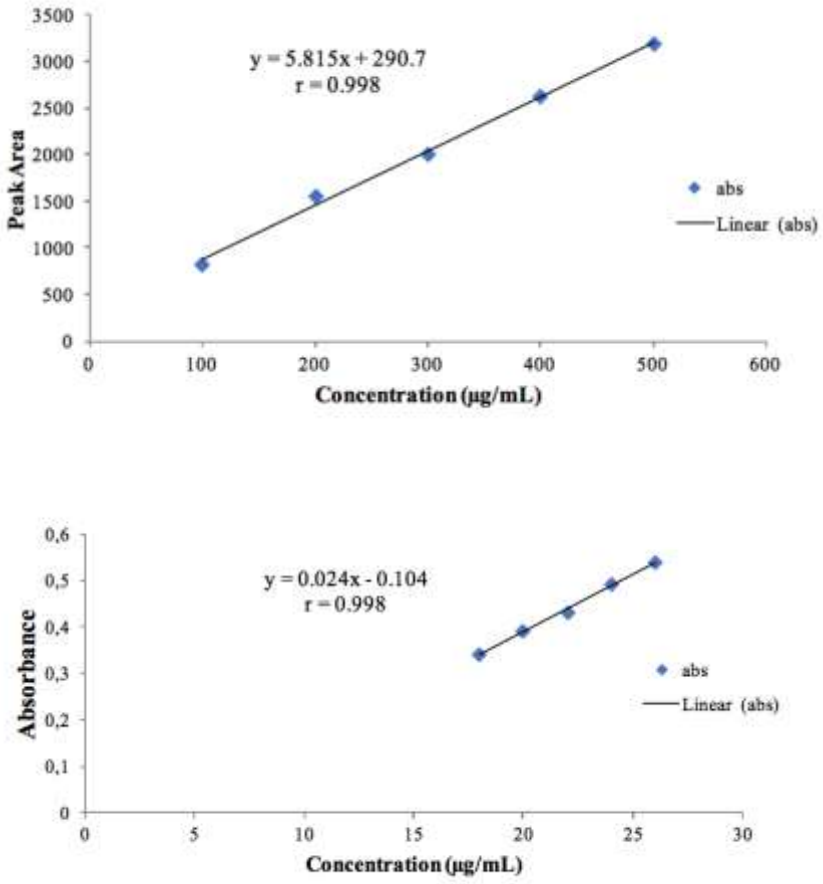


**Figure. 2. Standard calibration curves for thiamine using HPTLC-densitometry& UV-Vis spectrophotometric**

Thiamine contents were measured by using HPTLC and UV/Vis spectrophotometry methods and calculated in 5 grams of green beans which were 0.0396 and 1.0009 %, respectively. Thiamine contents of green beans are presented in figure 3. From the result of the study, thiamine content found in green beans using HPTLC-densitometrymethod was lower than the UV / Vis Spectrophotometry method.

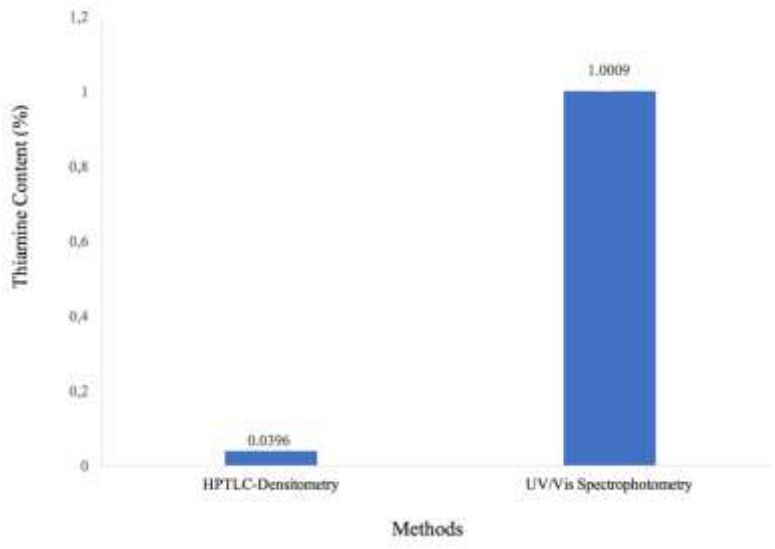


**Figure 3. Thiamine content (%) in 5 grams of green beans**

Results of analysis of the studied component by the proposed methods were compared statistically with those obtained by reported HPTLC-densitometry and UV/Vis spectrophotometry method. The methods showed a significant difference between them using student's-t and F- ratio tests with sig. = 0.008 (< 0.05).Many factors may cause thiamine levels obtained by the HPTLC-densitometry method is smaller than UV/Vis spectrophotometry method, more steps in sample preparation, sample bottling on plate and room temperature while working will affect the thiamine assay. Thiamine is unstable and easily degraded at high temperature and it is possible that some of thiamine was lost during sample preparation. Thiamine is also sensitive to oxidation by ROS generated by UV light and ionizing radiation[3].

## Conclusion

HPTLC-densitometry and UV-Vis spectrophotometry have been investigated for thiamine analysis. These methods are simple, flexible, cost-effective, accurate and present the advantage of the simultaneous processing of standards and samples such as short analysis time and low solvent consumption.The results of the analysis using the proposed HPTLC-densitometry and UV-Vis spectrophotometry method showed a significance difference statistically. However, the two proposed methods were successfully applied to the determination of thiamine in green beans and can be employed for the routine and large-scale analysis of thiamine in green beans and other samples.

## Acknowledgments

This work was supported by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia.

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