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Analytical Methods

# Measurement of caffeine in coffee beans with UV/vis spectrometer

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## Abstract

In this research work using UV/vis spectrophotometer the molar decadic absorption coefficients and transitional dipole moment of pure caffeine in water and dichloromethane were obtained at 272 and 274.7 nm. The molar decadic absorption coefficients of caffeine in water and dichloromethane at these wavelengths are 1115 and 1010 $m^2 mol^{-1}$, respectively. The calculated values for the transitional dipole moment of caffeine in water and in dichloromethane are $10.40 \times 10^{-30}$ and $10.80 \times 10^{-30}$ C m, respectively. After characterizing caffeine in water and dichloromethane, fast and simple methods were developed that enable to quantify the content of caffeine in coffee beans. The methods helped in extracting caffeine from coffee dissolved in water by dichloromethane, and Gaussian fit was applied to eliminate the possible interference with the caffeine spectra.

*Keywords:* Absorbance; Caffeine; Characterization; Extraction; UV/vis spectrophotometer

## 1. Introduction

Caffeine is found in various kinds of foods and drinks that we consume in daily life (Singh & Sahu, 2006). It causes various physiological effects such as relaxation of bronchial muscle, stimulation of the central nervous system, gastric acid secretion and dieresis Bolton and Null (1981). And their concentration in vivo is a key mark for various disorders including heart disease, carcinogenesis, kidney malfunction and asthma (Zhang, Lian, Wang, & Chen, 2005). On the other hand, chemical analysis of caffeine in coffee beans is also used as an additional tool for evaluating coffee quality. Higher caffeine contents associated with highest quality samples compared to other Arabic samples have been reported by Franca, Mendonca, and Oliveira (2005). Therefore, establishing a rapid and cheap analytical method for the determination of caffeine in coffee beans has an interest for a wide range of physiological effects on the human body and quality controls.

Several chemical and physical methods have been developed for the determination of caffeine in coffee and other beverages. The most widely used methods for the determination of caffeine in beverages include various analytical techniques such as derivative spectrophotometer (Alpdogan, Karbina, & Sungur, 2002) HPLC (Branstrom & Edenteg, 2002; Casal, Oliveira, & Ferreira, 2000; Minawlsawa, Yoshida, & Takali, 2004; Ortega-Burrales, Padilla-Weigand, & Molina-Diaz, 2002), Fourier Transform infrared (Bousain, Garriques, Garriges, & Guardia, 1999; Najafi, Hamid, & Afshin, 2003; Paradkar & Irudayaraj, 2002), NIR reflectance spectrometry (Chen, Zhao, Huang, Zhang, & Liu, 2006), Raman spectroscopy (Edawards, Munish, & Anstis, 2005) and capillary electrophoresis (Zhang et al., 2005), which have been reported. Although Spectrophotometer is a fast and simple method it is not possible to determine caffeine directly in coffee beans by conventional UV absorption measurement due to the spectral overlap (Zhang et al., 2005). On the other hand, the derivative spectrophotometer is relatively easy; however, it is not reliable for the determination of small concentration of caffeine in samples. With HPLC methods the use of expensive equipments and the demand for more operator attention prevent

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their application in small industrial laboratories where only a few analyses are performed each day (Alpdogan et al., 2002; Zhang et al., 2005). Other methods such as FT infrared, Raman and NIR reflectance spectrometry are equally versatile for the measurement of caffeine and do not require expensive chemicals, however, such instruments are expensive and are not available in most laboratories.

In this paper, a method for measuring caffeine content in Arabic coffee beans of Ethiopia is reported using UV/vis spectrophotometer, which is available in most laboratories. Moreover the methods are easy, fast and cheap for the determination of the caffeine contents in coffee beans. The methods include characterizing pure caffeine in water and dichloromethane, and based on these several techniques were developed to determine caffeine in coffee beans. These are extracting caffeine from coffee using dichloromethane solution. After caffeine is extracted from coffee using dichloromethane there still exist some interfering substances from chlorogenic acid related compounds (*p*-coumaroquinic acid) that have a maximum peak at a wavelength of 308–310 nm and these interfering matrices were eliminated by Gaussian fit.

## 2. Materials and methods of the experiment

### 2.1. Chemicals and samples

Dichloromethane (Aldrich, Germany), chloroform (Riedel–Detlaen) distilled and de-ionized water (AAU, Ethiopia) were used. A caffeine sample was obtained from Evan, England and Arabic coffee beans were provided by Ethiopia coffee and tea quality and liquoring center, Ethiopia, which were washed at the export standard of the center. The coffee samples were collected from the southern part of Ethiopia regions (the specific areas are Benchi maji, Gediyo yirgachefe, Tepi, Godere Goma limu, and Besema) without considering their varieties. Each coffee sample was measured five times and the mean value and standard deviation for each are presented in Table 1. The coffee samples used for these researches are green (unroasted) coffee beans.

### 2.2. Instrumentation

Different laboratory apparatus and UV/vis spectrophotometer with a model SPECTRONIC® GENESYS™ 2PC, and slit width 2 nm with quartz cuvette 1 cm. The spectrophotometer was interfaced with computer, which was operated by WinSpec™ application softwares.

### 2.3. Characterization of caffeine

For the characterization of caffeine de-ionized water and dichloromethane were used. A mass of $5.03 \times 10^{-4}$ g caffeine dissolved in 23.07 $\text{cm}^3$ de-ionized water ($c = 109.72 \times 10^{-3}\ \text{mol m}^{-3}$) and a caffeine of mass $3.05 \times 10^{-4}$ g dissolved in 15.19 $\text{cm}^3$ of dichloromethane ($c = 103.37 \times 10^{-3}\ \text{mol m}^{-3}$) were stirred for 1 h using magnetic stirrer. The absorbance of the solutions was measured by UV/vis spectrophotometer at room temperature. From the absorbance molar decadic absorption coefficient and transitional dipole moment of caffeine in water and dichloromethane were, respectively, obtained.

Table 1
The mean percentage of caffeine for five independent measurements for samples collected from different areas (origin) by UV/vis spectrophotometer

| Origin of the coffee samples | Mass of coffee in (mg) | Mass of caffeine in coffee (mg) | The percentage of caffeine in coffee (w/w%) | Mean values for five independent measurement ± deviation (w/w%) |
|---|---|---|---|---|
| Benchi maji | 49.80 | $5.40 \times 10^{-1}$ | 1.08 | $1.10 \pm 0.01$ |
| | 49.90 | $5.45 \times 10^{-1}$ | 1.09 | |
| | 51.90 | $5.84 \times 10^{-1}$ | 1.12 | |
| | 51.80 | $5.75 \times 10^{-1}$ | 1.10 | |
| | 52.00 | $5.72 \times 10^{-1}$ | 1.10 | |
| Gediyo yergachefe | 50.30 | $4.98 \times 10^{-1}$ | 0.99 | $1.01 \pm 0.04$ |
| | 50.50 | $4.90 \times 10^{-1}$ | 0.97 | |
| | 50.60 | $4.95 \times 10^{-1}$ | 0.99 | |
| | 50.80 | $5.32 \times 10^{-1}$ | 1.06 | |
| | 50.90 | $5.34 \times 10^{-1}$ | 1.05 | |
| Tepi | 49.90 | $5.43 \times 10^{-1}$ | 1.09 | $1.07 \pm 0.02$ |
| | 50.20 | $5.28 \times 10^{-1}$ | 1.05 | |
| | 50.20 | $5.31 \times 10^{-1}$ | 1.06 | |
| | 50.80 | $5.31 \times 10^{-1}$ | 1.05 | |
| | 50.90 | $5.50 \times 10^{-1}$ | 1.08 | |
| Godere | 50.20 | $5.90 \times 10^{-1}$ | 1.18 | $1.19 \pm 0.02$ |
| | 50.20 | $5.96 \times 10^{-1}$ | 1.19 | |
| | 50.3 | $6.49 \times 10^{-1}$ | 1.22 | |
| | 50.40 | $5.93 \times 10^{-1}$ | 1.18 | |
| | 50.70 | $6.00 \times 10^{-1}$ | 1.18 | |

### 2.4. Coffee sample preparation

Raw coffee were ground and screened through 250 μm sieve to get a uniform texture. An accurately weighed amount of sieved coffee (approximately 50 mg) was dissolved in 25 ml of distilled water. The solution was stirred for one hour using magnetic stirrer and heated gently to remove caffeine easily from the solution. In addition the solution was filtered by a glass filter to get rid of particle from solution.

### 2.5. Liquid–liquid extraction of caffeine

In this research for liquid–liquid extraction chloroform and dichloromethane were used for comparison. However, it had been observed that many interfering matrices were extracted with chloroform than dichloromethane, due to this dichloromethane was selected for the extraction. Literature report also indicated that even if both solvents were useful for decaffeinating caffeine from coffee beans according to Clarke, (1980), Rofti (1971) the current most widely used solvent for decaffeinating in coffee beans was dichloromethane. The efficiency of dichloromethane to extract caffeine from coffee beans is 98–99%.

Extraction has been done according to the following procedures. The coffee solution prepared above (under coffee sample preparation) was mixed with dichloromethane by volume ratio (25:25 ml) for the extraction of caffeine from coffee. First, a mixture of the solution was stirred for 10 min. Then, using separatory funnel caffeine was extracted by dichloromethane from the solution. The extraction of caffeine proceeded 4 times with 25 ml dichloromethane at each round. The caffeine extracted by dichloromethane at each round was stored in volumetric flasks. Finally, the absorbance of the solution was measured by UV/vis spectrophotometer in the range of 200–500 nm against the corresponding reagent blank. All glassware was thoroughly cleaned, rinsed with distilled water and dried before use.

### 2.6. Gaussian fit

In addition to caffeine spectra there are interfering bands from other coffee components extracted by dichloromethane and the peak of these bands was observed at the wavelength of 308–310 nm. The compounds attributed to this are known to be chlorogenic acid related compounds (*p*-coumaroquinic acid). It is clear that this interfering band has an effect on the maximum peak of caffeine. Therefore, in this research matrices were eliminated by Gaussian fit. The peak absorbance for calculating the concentration of caffeine was obtained after subtracting the Gaussian fit from the total caffeine spectra.

## 3. Results and discussion

### 3.1. UV–vis absorption of caffeine in water and dichloromethane

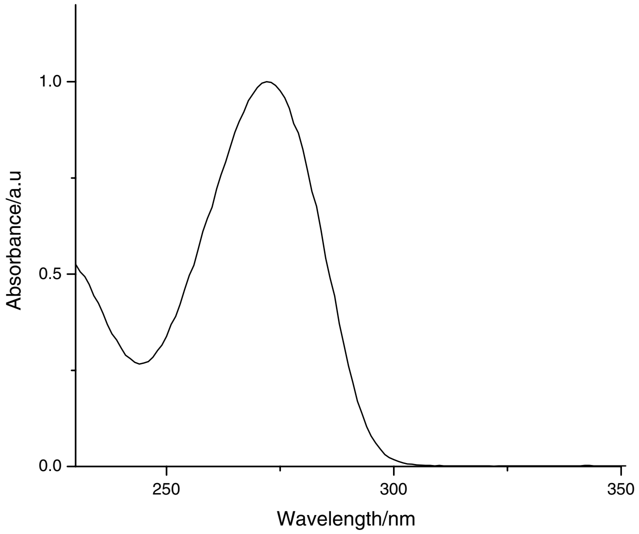

Fig. 1. UV–vis spectra of caffeine in water.

The UV–vis absorption spectrum of caffeine in water is found to be in the region of 243–302 nm at room temperature. It is clearly shown in Fig. 1 that the spectral intensity of caffeine drops to zero at wavelength greater than 302 nm, on the other hand a new peak absorbance is noticed at a wavelength below 243 nm. This new spectrum is expected to be the peak absorbance due to the solvent. The peak absorbance of the solution is found to be $A = 1.224$ at the maximum wavelength $\lambda_{\max} = 272$ nm. The maximum peak absorbance for caffeine observed in these experiments was quite similar to those reported by Clarke and Macrae (1985). The molar decadic absorption coefficient measuring the intensity of optical absorption at a given wavelength was calculated using Beer–Lambert's equation (Liptay, 1969). The molar decadic absorption coefficient of caffeine in water is computed and value of $\varepsilon_{\max} = 1115\ \mathrm{m^2\ mol^{-1}}$ is obtained. The transitional dipole moment of the dissolved molecule, which is related to the molar decadic absorption coefficient by the integral absorption coefficient, was calculated using the following equation (Liptay, 1969; Michale, 1999):

$$I_{\mathrm{A}} = \int_{\mathrm{band}} \frac{\varepsilon(\nu)}{\tilde{\nu}}\,\mathrm{d}\tilde{\nu} = \frac{1}{3}\,\frac{2h\pi^2 N_{\mathrm{a}}}{\ln(10)c_0\varepsilon_0}|\mu_{\mathrm{fi}}|^2 = \frac{1}{3}S|\mu_{\mathrm{fi}}|^2$$

where $S = 2.9352 \times 10^{60}\ \mathrm{C^{-2}\ mol^{-1}}$. Fig. 2 shows the spectra of caffeine in water given as a function of molar decadic absorption coefficient over wave number versus wave number ($\varepsilon(\nu)/\tilde{\nu}$ versus $\tilde{\nu}$). The integrated area under this curve $105.92\ \mathrm{m^2\ mol^{-1}}$ was obtained by integrating from $\tilde{\nu}_1 = 33{,}000\ \mathrm{cm^{-1}}$ to $\tilde{\nu}_2 = 41{,}000\ \mathrm{cm^{-1}}$ therefore, the transitional dipole moment of caffeine in water $\mu_{\mathrm{fi}} = 10.40 \times 10^{-30}$ C m was obtained.

Similarly, the UV/vis absorption spectra of caffeine in dichloromethane was found to be in the region of 243–301 nm. Its peak absorbance $A = 1.043$ was obtained at maximum wavelength $\lambda_{\max} = 274.7$ nm. The molar decadic

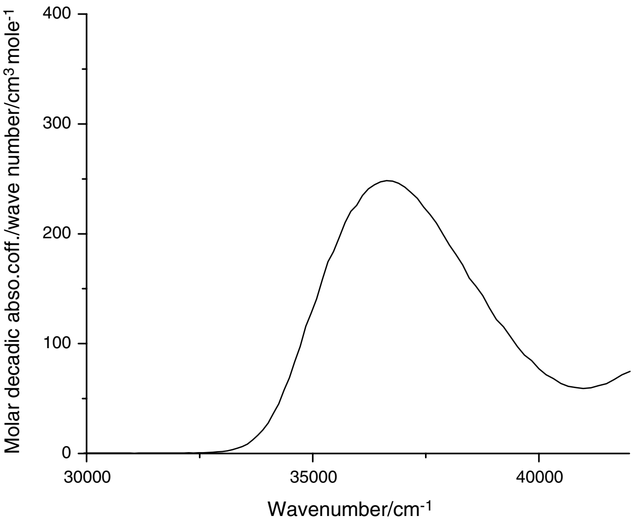

Fig. 2. $\frac{\varepsilon(\tilde{\nu})}{\tilde{\nu}}$ versus $\tilde{\nu}$ caffeine in water.

absorption coefficient of caffeine in dichloromethane $\varepsilon_{\max} = 1010\ \mathrm{m}^2\ \mathrm{mol}^{-1}$ is obtained. Fig. 3 shows the peak absorbance versus maximum wavelength of caffeine in dichloromethane. And Fig. 4 below shows the graph of $\varepsilon(v)/\tilde{v}$ versus $\tilde{v}$ in dichloromethane. The integrated area under this graph is $I_A = 114.16\ \mathrm{m}^2\ \mathrm{mol}^{-1}$ in the wave number between $\tilde{v}_1 = 33,000\ \mathrm{cm}^{-1}$ and $\tilde{v}_2 = 41,000\ \mathrm{cm}^{-1}$. From the integral absorption value the transitional dipole moment of caffeine in dichloromethane is determined to be $\mu_{\mathrm{fi}} = 10.80 \times 10^{-30}\ \mathrm{C\ m}$.

### 3.2. Determination of caffeine in coffee beans

A UV/vis spectrophotometer method cannot be used directly for the determination of caffeine in coffee seeds owing to the matrix effect of UV absorbing substances in the simple matrix (Ortega-Burrales et al., 2002; Zhang et al., 2005). This effect is also clearly seen in the spectral bands of caffeine in coffee seeds (Fig. 5) dissolved in water. Hence, it is not suitable to determine the percentage of caffeine in coffee seeds due to overlapping of these interfering bands. In order to overcome this difficulty coffee was first dissolved in water and caffeine extracted from solution using dichloromethane. Dichloromethane is the most commonly employed method for extraction of caffeine from green coffee beans (Rofti, 1971). Many commercial products applied dichloromethane for decaffeinating the coffee beans for its extraction efficiency 98–99% (Clarke, 1980). The extraction was made four times until the spectrum of caffeine becomes flat when seen under UV/vis spectrophotometer. Fig. 6 below shows peak absorbance versus wavelength of caffeine spectra extracted using dichloromethane

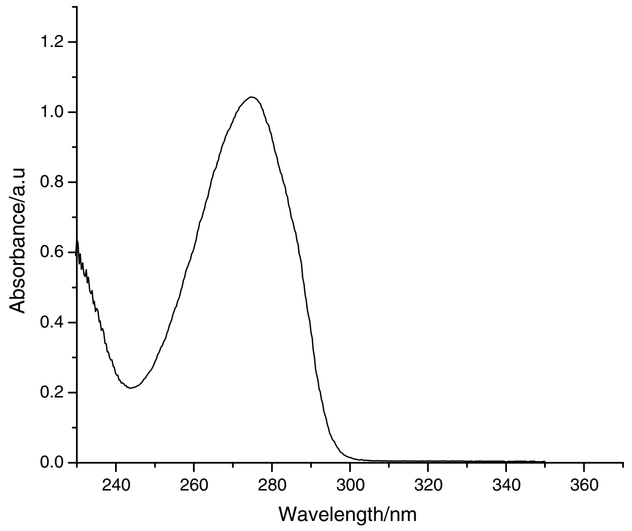

Fig. 3. UV–vis spectra of caffeine in dichloromethane.

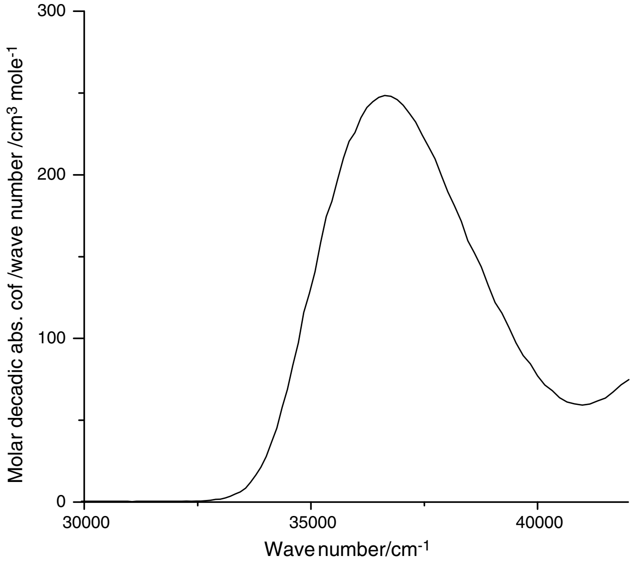

Fig. 4. $\frac{\varepsilon(\tilde{v})}{\tilde{v}}$ versus $\tilde{v}$ caffeine in dichloromethane.

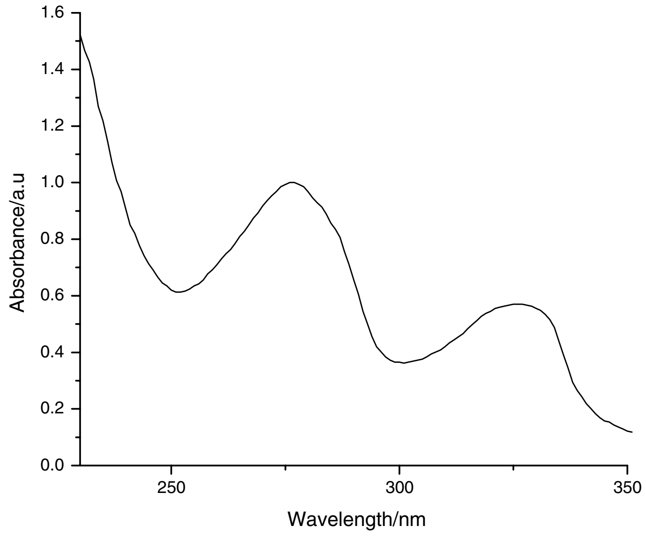

Fig. 5. UV/vis spectra of caffeine in coffee dissolved in water.

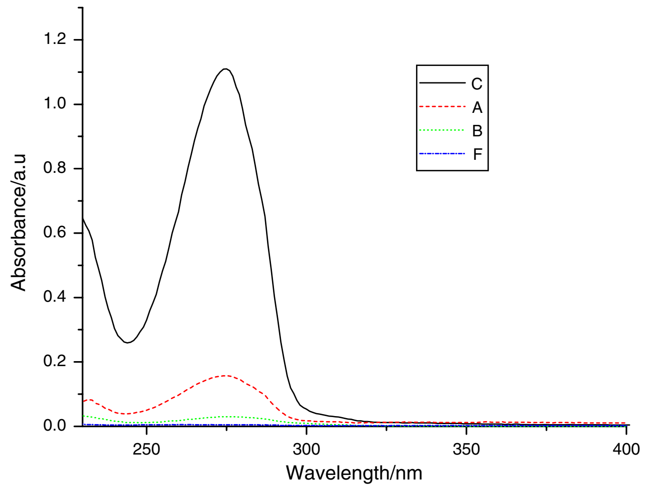

Fig. 6. The over lapped spectra of caffeine for different round of extraction (C) for first round, (A) for second round, (B) for third round and (F) for fourth round.

from water solution at different stages. For comparison the spectra of each stage were overlapped. It is clearly shown in Fig. 6 that the concentration of caffeine at each stage is different, hence the size of peak absorbance also varies. From the results obtained high amount of caffeine concentration was extracted in the first stage of extraction. Moreover as seen from the graph almost no caffeine peak was seen in the four rounds of extraction.

The extraction techniques could not completely remove the possible interferences with caffeine spectra. Therefore, extracting qualitative and quantitative information from the spectra composed of unresolved bands is impossible. The interfering bands were observed at wavelength 308 nm as shown in Fig. 8. From the literature report the compounds that absorbed in these regions are belong to chlorogenic acid related compounds (*p*-coumaroyquinic acid) (Martin, 1970; Clarke et al., 1985). It has been reported that the chlorogenic acid makes complexes with Caffeine. To eliminate these interference spectra Gaussian fit was applied. It is assumed that the line shape of interferences spectra has a Gaussian function. Fig. 7 shows the spectra of caffeine in coffee seeds extracted by dichloromethane before Gaussian fit while Fig. 8 shows the spectra of caffeine with Gaussian fit and Fig. 9 the spectra of caffeine after the best Gaussian fit is subtracted. As it is seen in Fig. 9, the line shape of the spectra after Gaussian fit is subtracted exactly similar to the shape of spectra of the pure caffeine dissolved in dichloromethane.

Further to compare this spectra with pure caffeine the two spectra were overlapped. Fig. 10 shows the normalized

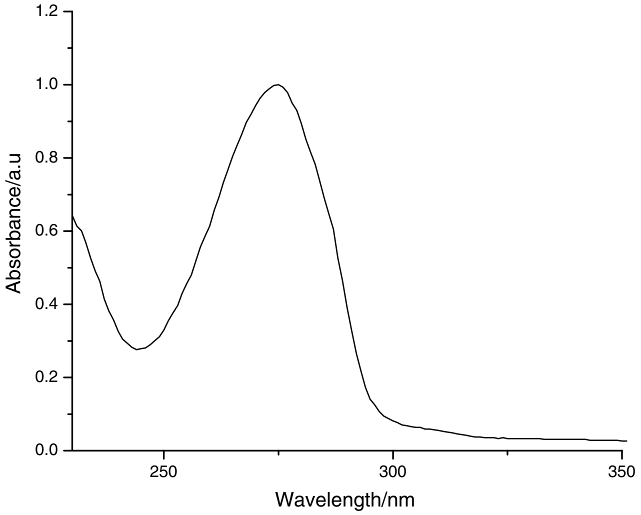

Fig. 7. UV–vis spectra of caffeine extracted by dichloromethane before Gaussian fitting.

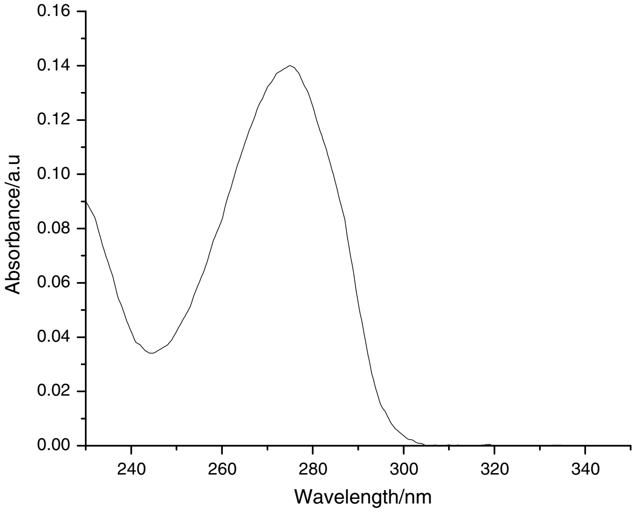

Fig. 9. The UV/vis spectra of caffeine after Gaussian fit subtracted. After Gaussian fit the spectra are similar with caffeine spectra dissolved in dichloromethane.

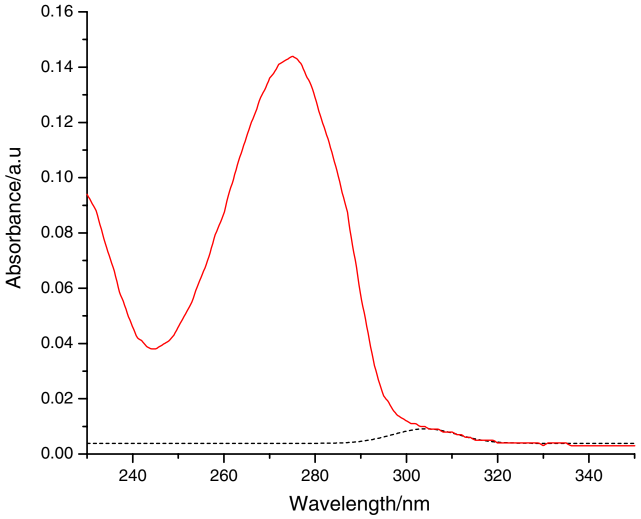

Fig. 8. The UV/vis (—) spectra of caffeine in coffee and (...) Gaussian fit. In this case the Gaussian fit is to eliminate peak found in the region of 308–310 nm.

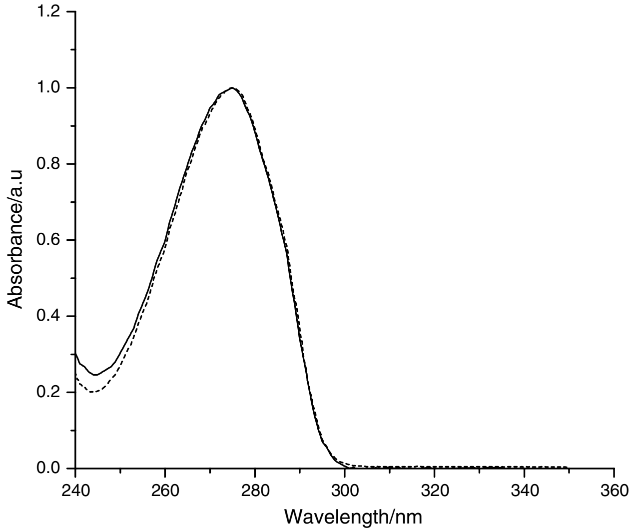

Fig. 10. It shows the normalized spectra of caffeine. (—) the spectra of pure caffeine and (...) the spectra of caffeine after Gaussian fit.

Table 2
Percentage of caffeine obtained by HPLC for coffee samples collected from different regions

| Origin of coffee samples | Percentage of caffeine (%) |
|---|---|
| Benchi Maji | 1.10 |
| Gediiyo yigachefe | 1.10 |
| Gomma Limu | 1.00 |
| Besema | 0.90 |

spectra of pure caffeine and the spectra after Gaussian fit; as is observed in the figure, the two spectra exactly fit each other. Therefore using extraction followed by Gaussian fit it is possible to get a good-resolved spectra of caffeine, which could make to determine the concentration of caffeine in coffee beans.

Using the proposed methods the percentage of caffeine in coffee beans for four samples was determined. This is simple for comparing the percentage of caffeine contents for samples collected from different Ethiopian regions. The samples were collected from Ethiopia coffee, tea quality and liquoring center to get a coffee treated under the same condition. All coffees are green and washed at the standard of the center. The mean percentage of caffeine in coffee seeds investigated using UV/vis spectrophotometer for five independent measurements are $1.1 \pm 0.01\%$ for Benchi maji, $1.01 \pm 0.04\%$ for Gediyo yirgachefe, $1.07 \pm 0.02\%$ for Tepi and $1.19 \pm 0.02\%$ for Godere, respectively, and shown in Table 1. On the other hand for comparison with HPLC, which is powerful in quantification the samples were analyzed and similar results were obtained. The summarized results of HPLC are shown in Table 2. The literature report also indicated by Farah, Monteiro, Calado, Franca, and Trugo (2006) also indicated that the highest caffeine content observed in Arabic green coffee beans was $1.23 \pm 0.06\%$ and the lowest was $0.96 \pm 0.01\%$. Moreover, it was also proposed by Illy (2002) that the percentage of caffeine for Arabic coffees was on the average less than 1.5%. Using derivative spectrophotometer it was also reported by Alpdogan et al. (2002) that the percentage of caffeine in coffee seeds was $1.36 \pm 0.03\%$.

## 4. Conclusion

The current methods developed on UV/vis spectrophotometer are relatively easy, fast, cheap and highly sensitive for the determination of caffeine content in coffee beans. Moreover chemicals and equipments necessary to carry out the analysis by proposed methods are those which are available in most common laboratories. In addition the methods have been tested for roasted coffee and are applicable without any restriction.

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