



Determination of Caffeine in Coffee Samples by High Performance Liquid Chromatography and Ultra Violet - Visible Spectrophotometry Methods from Wollega, Ethiopia

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Abstract: In this research caffeine content in coffee sample from Abe Dongoro, Sasiga, Gida Ayana and Siburu Sire of Wollega administrative zone of Ethiopia were determined using High Performance Liquid Chromatography (HPLC) and UV-Vis Spectrophotometry methods. Caffeine in aqueous extract of coffee samples was extracted with dichloromethane prior to analysis by UV-Vis spectrophotometry method and dichloromethane was evaporated from the extract and the extract was dissolved in water (HPLC grade) to determine caffeine contents in coffee samples using HPLC method. The linearity of the HPLC and UV-Vis spectrophotometry methods were $R^2 = 0.9999$ and $R^2 = 0.9997$ respectively. HPLC and UV-Vis spectrophotometry methods were found to be accurate with recoveries of 97.5% and 117.4%, respectively. Limits of detection (LOD) obtained were 0.148 mg/L for HPLC method and 0.284 mg/L for UV-Vis spectrophotometric method. The caffeine contents in coffee samples obtained using UV-Vis spectrophotometry method was 3.42, 2.638, 2.207 and 2.986 mg/L for Abe Dongoro, Gida Ayana, Sasiga and Siburu Sire coffee samples respectively. Similarly, using HPLC method the caffeine contents in coffee samples obtained was 1.871, 1.601, 1.307, 1.83 mg/L for Abe Dongoro, Gida Ayana, Sasiga and Siburu Sire coffee samples. There is a significant difference between the caffeine contents in coffee samples obtained by the two methods.

Keywords: Coffee Samples, Caffeine, UV-Vis Spectrophotometry, High Performance Liquid Chromatography, Horro Guduru Wollega, East Wollega

1. Introduction

Coffee is one of the popular beverages that is widely used around the world due to its pleasant flavor, aroma and fitness benefits. Botanically it belongs to genus *Coffea* in the *Rubiaceae* family. Genus *Coffea* contains over 80 diverse numbers of species. However, *Coffea arabica* and *Coffea robusta* are the main genus *Coffea* species [1]. Arabica coffee is grown-up in about tropical and sub-tropical of 80 countries. Ethiopia is among these countries, which heavily depend on coffee exports for foreign exchange earnings [2].

Coffee has a complex mixture of chemical constituents such as phenolic compounds, caffeine, trigonelline, chlorogenic acids, protein, lipids and minerals [3-5]. The chemical content of coffee varies with species, geographical source, climatic parameters, cultivation system (organic

versus conventional system) [6].

Caffeine is a natural alkaloid widely used in food industry as a psycho stimulant in beverages and foods for information processing and cognitive performance [7]. It is found in tea [8], chocolate [9], caffeinated, decaffeinated beverages and energy drinks [10], Arabica coffee [11] and Robusta coffee [12].

Caffeine is used to increase alertness, improves short-term memory and increases the efficiency of certain drugs.

However, over dose use of caffeine is a matter of health concerns such as gastric acid secretion and higher risk of miscarriage [13]. Therefore, monitoring of caffeine content in food and beverages is an important challenge of food quality control.

Various analytical and electro analytical techniques such as high performance liquid chromatography [14, 15], Fourier

transforms near infrared reflectance spectroscopy [16], UV-Vis spectrophotometry [17, 18] and voltammetry [19] are reported for the determination of caffeine contents in food and beverages.

Caffeine contents in coffee samples grown at different part of Ethiopia were studied using several analytical techniques. However, to this date, there is no published research finding that made use of Ultra Violet-Visible Spectrophotometry (UV-Vis) and High Performance Liquid Chromatography (HPLC) for the determination of the caffeine contents in coffee samples from Eastern and Horro Guduru Wollega Zones in the Western part of Ethiopia. Therefore, the objective of this study was to determine caffeine in coffee samples from *Abe-Dongoro*, *Sasiga*, *Gida-Ayana* and *Sibu-Sire* districts in Wollega zones of Western Ethiopia by High Performance Liquid Chromatography (HPLC) and Ultra Violet-Visible Spectrophotometry (UV-Vis) methods.

2. Experimental

2.1. Study Area

Coffee bean samples were collected from three selected districts of Eastern Wollega administrative zone from the *Sasiga*, *Gida-Ayana*, *Sibu-Sire* and *Abe-Dongoro* district of *Horro-Guduru* Wollega administrative zone directly from the farmers' farmlands. The geographical maps of the sampling sites were displayed in Figure 1. The districts were preferred purposely as they are major coffee producing parts of the administrative zone.

2.2. Chemicals and Reagents

Chemicals and reagents used were of analytical grade; standard caffeine powder (Aldrich, Germany), HPLC grade methanol (Aldrich, Germany), HPLC grade water and dichloromethane (Aldrich, Germany).

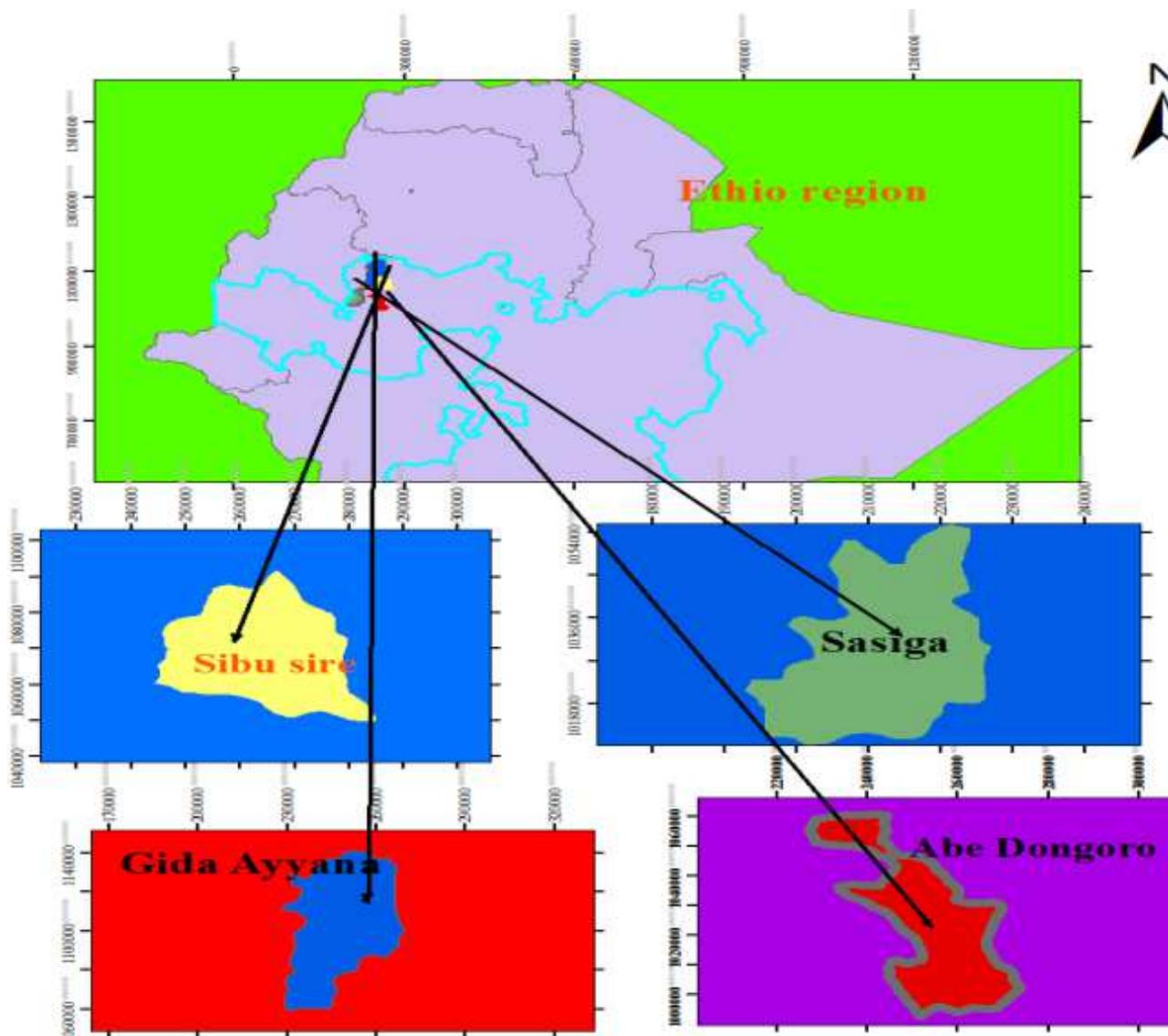


Figure 1. Geographical location of sampling site.

2.3. Instruments

Caffeine contents in coffee samples was determined using UV-Vis spectrophotometry (18- 1884-01-0076, spectral bandwidth 2.00 nm) and HPLC system (Agilent1260, Germany) equipped with a pump (G1310B), column (G1316A/ Agilent poroshell-C₁₈ (4.6 x 250 mm, 2.7 μ m)), auto sampler (G1329B), variable wavelength detector (G4286B) and Chemstation software.

2.4. Preparation of Standard Solutions

For analysis by HPLC, the stock solution of standard caffeine (1000 mg/L) was prepared by dissolving 100 mg of standard caffeine in 50 mL warm, ultrapure water in 100 mL of volumetric flask and filled to the final volume with ultrapure water. Intermediate standard solution of caffeine (100 mg/L) was prepared from the stock solution. Finally, concentrations of 2, 4, 8 and 16 mg/L caffeine solution were used to construct calibration curve.

For the UV-Vis spectrophotometric analysis, the stock solution of standard caffeine (1000 mg/L) was prepared by dissolving 100 mg of standard caffeine in 50 mL dichloromethane in 100 mL of volumetric flask and filled to the final volume with dichloromethane. Intermediate standard solution of caffeine (100 mg/L) was prepared by diluting the stock solution with dichloromethane. Finally concentrations of 0, 2, 4, 8 and 16 mg/L were prepared by diluting the working standard for calibration. For the quantitative determination of caffeine contents in coffee samples the λ_{max} (272 nm) of caffeine standard was selected from the reported literature, this is because of the characteristics observed peak of caffeine at 271–276 nm [20, 21].

2.5. Coffee Sample Preparation

A 20 g of coffee bean from each sample was roasted by using the conventional coffee roasting machine. After roasting, the coffee bean samples were cooled at room temperature. Each of the roasted and cooled coffee bean samples was grounded and homogenized using an electric coffee grinder machine. After that, each of the coffee powder was screened through 300 μ m sieve to get a uniform mixture and kept in plastic bag at room temperature until it is used for the extraction. The extraction of caffeine from the aqueous solution into dichloromethane was carried out by the reported methods [22, 23].

Trigonelline, chlorogenic and caffeic acids are the primary interference in the quantitative determination of caffeine in coffee samples using UV-Vis spectrophotometry method. In order to overcome this difficulty the coffee samples was first dissolved in water and then the caffeine was extracted from the aqueous extract using dichloromethane. The efficiency of dichloromethane to extract caffeine from coffee beans is 98–99% [23]. For HPLC analysis, 2 mL aliquot from dichloromethane extract was pipetted into test tube and the dichloromethane was evaporated; the residue was dissolved in 2 mL HPLC grade water.

2.6. Chromatographic Analysis

The samples and standard solutions of 20 μ L were injected into the column with a flow rate of 0.8 mL/min using a mobile phase consisting of water and methanol (75:25, V/V). The column temperature was kept at 30 °C and data rate at 10 Hz. Chromatographic data for caffeine were collected at 272 nm.

2.7. Method Validation

Linearity of the calibration curves was evaluated based on the magnitude of the regression coefficient (R^2). Accuracy was validated by using spike-recovery method. The limit of detection (LOD) and limit of quantification (LOQ) of the analytical method is obtained based on the standard deviation of response and slope of the calibration curve [24]. In this study, LOD and LOQ of the methods were calculated as $LOD=3.3 \sigma/s$; $LOQ=10 \sigma/s$, where, s is the slope of the calibration curve and σ is the standard deviation of the y-intercept of the regression line. Differences between groups were assessed by one-way analysis of variance (ANOVA). The results with $P < 0.05$ were regarded to be statistically significant.

3. Result and Discussion

3.1. Analytical Performance Characteristics

The performance parameters for both HPLC and UV-Vis spectroscopic analysis are presented in Table 1. The calibration curve for both of the analysis methods showed an excellent linear fit. LOD for HPLC and UV-Vis methods were 0.148 and 0.284 mg/L, respectively. Recoveries of both methods were satisfactory. Thus, the proposed methods were appropriate for determination of caffeine contents in coffee samples.

Table 1. Performance parameters of the HPLC and UV-Vis spectrophotometry methods.

| No | Parameters | HPLC method | UV-Vis method |
|----|----------------------------|-------------|---------------|
| 1 | Coefficient of correlation | 0.9999 | 0.9997 |
| 2 | LOD (mg/L) | 0.148 | 0.284 |
| 3 | LOQ (mg/L) | 0.449 | 0.86 |
| 4 | Recovery | 97.5% | 117.4% |

3.2. Quantity of Caffeine in Coffee Samples

The identity of the analyte was determined by comparing the retention time extracted from the coffee samples with retention time of standard caffeine. Under the optimized experimental condition, the retention time of caffeine obtained was 10.6 minutes. In addition, the identity of caffeine was confirmed by spiking one of the coffee extract with standard caffeine. An increase in peak area after caffeine standard addition to the coffee extract supported the identification of the caffeine extracted from the samples. The extracts of the coffee samples were analyzed using HPLC method to determine the caffeine contents in the samples (Table 2).

Table 2. Caffeine content in coffee samples obtained using HPLC method.

| Coffee samples | Retention time | Peak area | Concentration (mg/L) | % of Caffeine (w/w) |
|-----------------------------|----------------|-----------|----------------------|---------------------|
| Abe Dongoro | 10.649 | 179.856 | 1.871 | 1.12 |
| Gida Ayana | 10.627 | 156.336 | 1.601 | 0.96 |
| Sasiga | 10.637 | 130.671 | 1.307 | 0.78 |
| Sibu Sire | 10.637 | 176.324 | 1.830 | 1.1 |
| Sasiga (duplicated) | 10.616 | 130.157 | 1.301 | 0.78 |
| Gida Ayana (4000 mg spiked) | 10.490 | 212.578 | 2.246 | 1.35 |

The caffeine content in coffee samples obtained using HPLC method was in the range of 0.78- 1.12% (w/w). The highest caffeine content was obtained in the Abe Dongoro coffee sample which gave a concentration of 1.871 mg/L or 1.12% (w/w), followed by a Sibu Sire coffee sample which gave a concentration of 1.830 mg/L or 1.10% (w/w). The least caffeine content was obtained in Sasiga coffee sample which gave a concentration of 1.307 mg/L or 0.78% (w/w) (Table 2). The results of the present study are comparable

with the results reported in the literature 0.06-2.55% (w/w) [25], 0.84-1.15% (w/w) [26] and 0.8-1.4% (w/w) [27] for caffeine in coffee samples.

The absorbance of the caffeine in each coffee sample solution was measured using UV-Vis spectrophotometry at 272 nm against the corresponding blank (dichloromethane). Caffeine content in coffee samples obtained by UV-Vis spectrophotometry method was presented in Table 3.

Table 3. Caffeine content in coffee samples obtained by UV-Vis spectrophotometry method.

| Coffee samples | Conc.(mg/L) (mean ± sd) | % of Caf (w/w) (mean ± sd) |
|-----------------------------|-------------------------|----------------------------|
| Abe Dongoro | 3.420±0.059 | 2.052±0.036 |
| Gida Ayana | 2.638±0.026 | 1.583±0.015 |
| Sasiga | 2.207±0.009 | 1.322±0.005 |
| Sibu Sire | 2.986±0.016 | 1.792±0.01 |
| Gida Ayana (4000 mg spiked) | 3.420±0.076 | 2.052±0.046 |

The highest caffeine content was obtained in the Abe Dongoro coffee sample which gave a concentration of 3.420 mg/L or 2.052% (w/w), followed by a Sibu Sire coffee sample which gave a concentration of 2.986 mg/L or 1.792% (w/w). The least caffeine content was obtained in Sasiga coffee sample which gave a concentration of 2.207 mg/L or 1.322% (w/w).

The caffeine content in coffee samples obtained using UV-Vis spectrophotometry method was in the range of 1.322 - 2.052% (w/w). There was a significant difference ($p < 0.05$) in caffeine contents among all the coffee samples. The results of the present study are comparable with the caffeine

contents reported in the literature 0.9-3.01% (w/w) [21], 1.24-2.54% (w/w) [28] for caffeine in coffee samples.

The caffeine content in coffee samples obtained using UV-Vis spectrophotometry method was compared with the caffeine content in coffee samples obtained using HPLC method (Table 4). In contrast to this, the UV-Vis spectrophotometry method is much faster than the HPLC method. The caffeine contents in coffee samples were found in the range of 1.322 - 2.052% (w/w) using UV-Vis spectrophotometry method and 0.78 - 1.12% (w/w) using HPLC method.

Table 4. Comparison of caffeine content in coffee samples% (w/w) obtained using UV-Vis spectrophotometry and HPLC methods.

| No. | Sample name | Method | |
|-----|-------------|--------|------|
| | | UV-Vis | HPLC |
| 1 | Abe Dongoro | 2.052 | 1.12 |
| 2 | Gida Ayana | 1.583 | 0.96 |
| 3 | Sasiga | 1.322 | 0.78 |
| 4 | Sibu Sire | 1.792 | 1.1 |

The present results indicates that the caffeine contents in coffee samples obtained using UV-Vis spectrophotometry method was higher than the caffeine contents in coffee samples obtained using HPLC method.

Application of statistical analysis (paired t-test at 0.05

levels) to the data obtained by the two methods indicated that there is significant difference between them. The caffeine contents of coffee samples obtained in this study was compared with caffeine values reported for coffee samples from different parts of the Ethiopia (Table 5).

Table 5. Comparison of results of the present study with the reported literature

| Method | % Caf (w/w) | Origin of coffee | References |
|--------|-------------------|---------------------|--|
| UV-Vis | 0.97 - 1.53 (n=4) | North West Ethiopia | Belete Tewabe & Solomon Libsu, 2015 [29] |
| UV-Vis | 0.6 - 0.9 (n=16) | Hararghe | Ephrem Demissie et al., 2016 [30] |
| HPLC | 0.6 - 1.1 (n=9) | South West Ethiopia | Mulu Hagos et al., 2018 [22] |
| HPLC | 0.62 - 1.99 (n=5) | Gojjam | Maria et al., 2000 [31] |

| Method | % Caf (w/w) | Origin of coffee | References |
|--------|--------------------|------------------|---------------------------------|
| HPLC | 1.31-1.36 (n=6) | Bale | Legesse Adane et al., 2018 [32] |
| UV-Vis | 1.32 - 2.052 (n=4) | *Wollega | Present work |
| HPLC | 0.78 - 1.12 (n=4) | *Wollega | Present work |

* Wollega = Eastern and Horro Guduru.

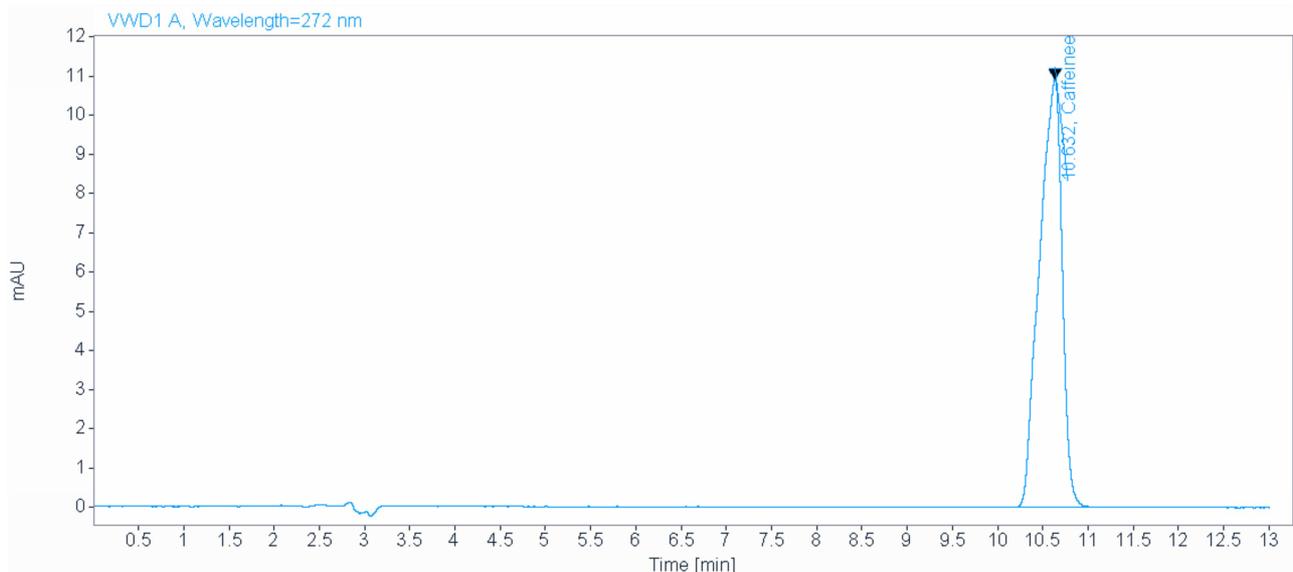
The caffeine content in coffee samples reported in the present study using UV-Vis spectrophotometry method is slightly greater than the reported caffeine contents in North West Ethiopia and Hararghe coffee samples using UV-Vis spectrophotometry method and slightly greater than the reported caffeine contents of South West Ethiopia, Gojjam and Bale coffee samples using HPLC method.

The obtained caffeine content in coffee samples in this study using HPLC method is slightly smaller than caffeine contents in Gojjam coffee samples and comparable with the South West Ethiopia coffee samples reported by HPLC method and somewhat smaller than the caffeine contents in North West Ethiopia coffee samples reported using UV-Vis spectrophotometry method.

In general the caffeine contents in coffee samples obtained in the present work is in the range of caffeine contents of export standard Ethiopian coffee samples (0.46-2.82% w/w) reported using HPLC method [31]. The difference in caffeine contents in coffee samples are due to several factors such as coffee variety, genetic properties of the cultivars, maturity of the beans at harvest, harvesting method and postharvest processing conditions (fermentation, washing, drying, storage), agricultural practices (shade, pruning, fertilization), environmental factors (soil, altitude, sun exposure), climatic parameters (rainfall, temperature), method of preparation (the brewing of coffee) and analytical methods used for the determination of caffeine in coffee samples [6, 33].

Appendix

Appendix A: Chromatogram of 2 mg/L of Standard Caffeine

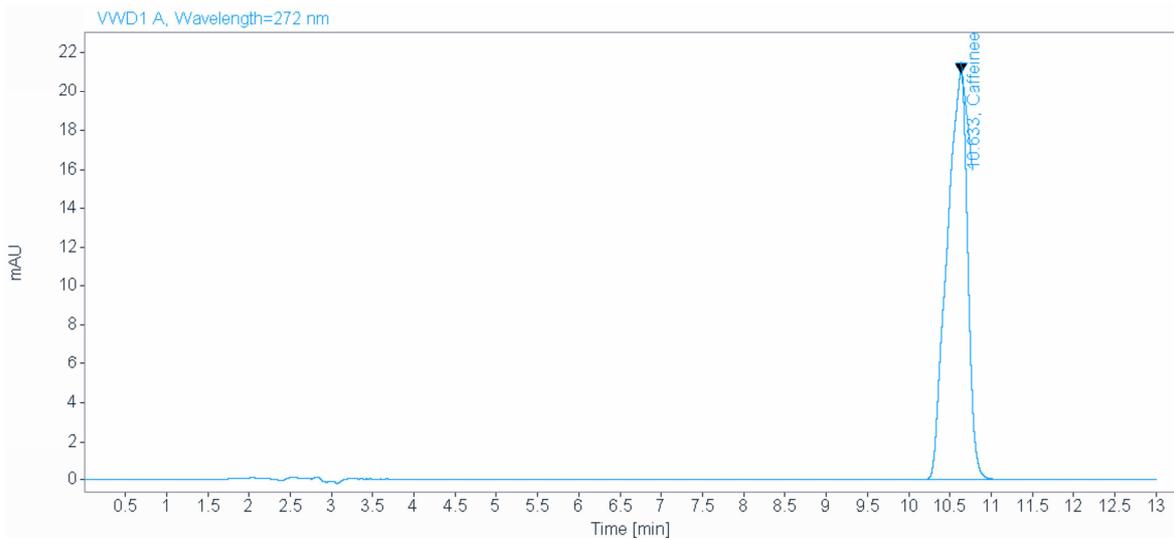
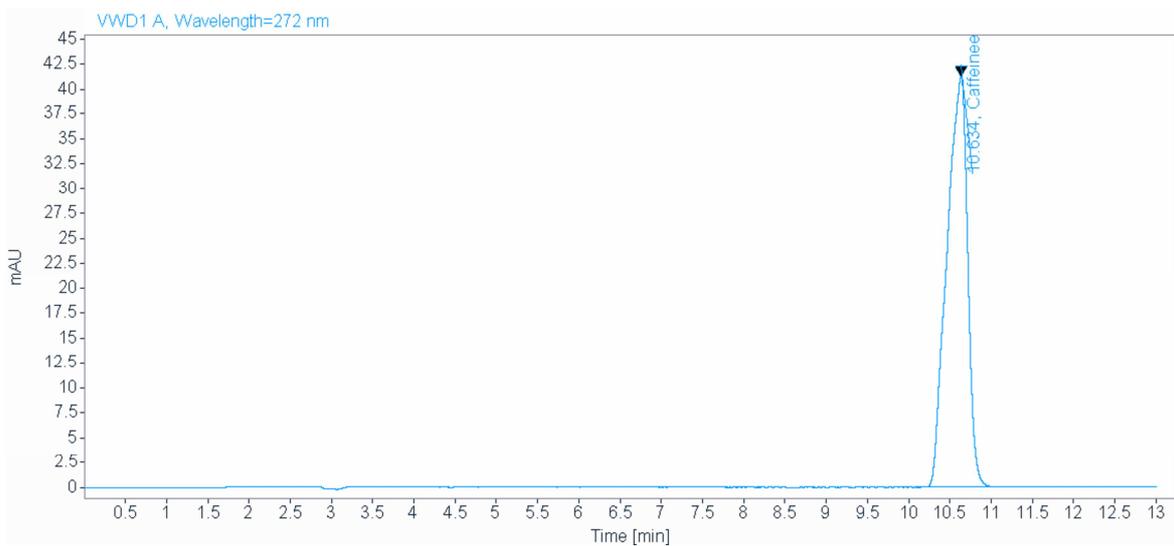
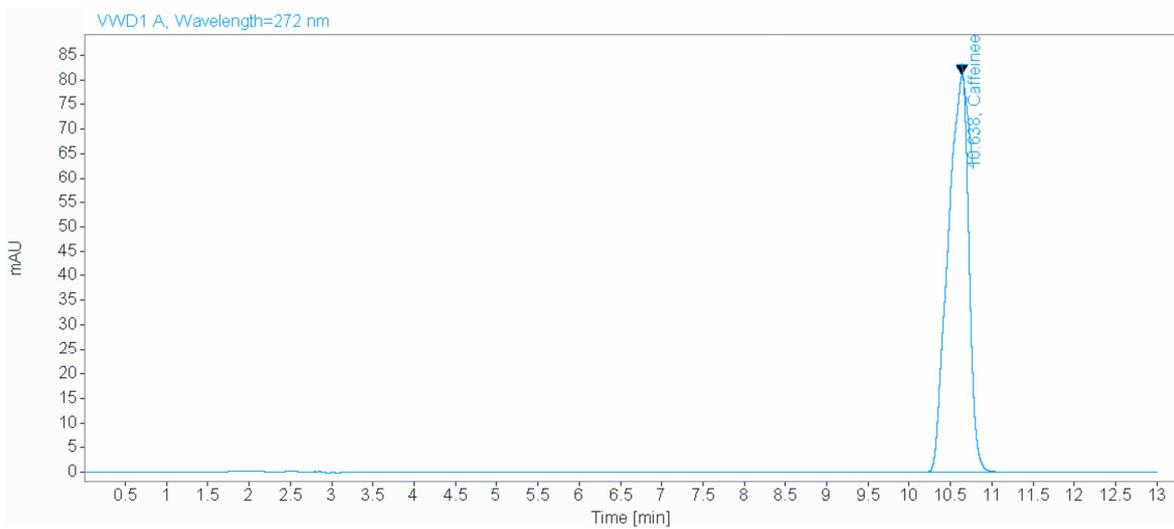


4. Conclusion and Recommendations

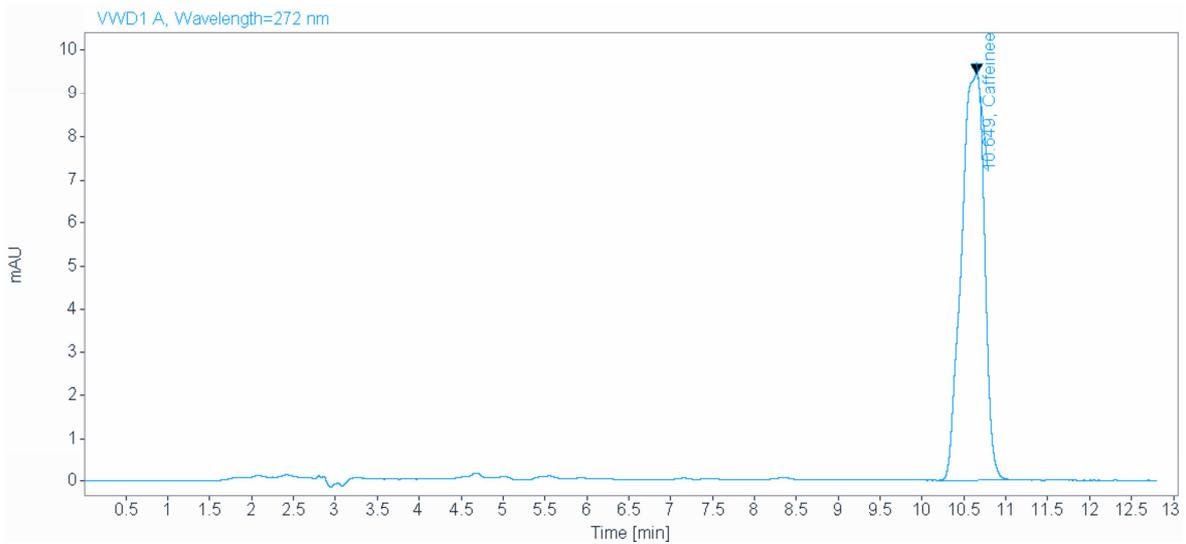
In this study, HPLC and UV-Vis spectrophotometry methods were successfully applied for the determination of caffeine contents in coffee samples grown in Wollega from Sasiga, Gida Ayana, Sibbu Sire and Abe Dongoro woredas. Variations of the caffeine contents in coffee samples were observed; these variations depend on the geographical origin of the coffee samples and the analytical methods used for caffeine content determination in coffee samples. Significant difference was observed between the caffeine value obtained by HPLC and UV-Vis spectrophotometry methods. The caffeine content in coffee samples obtained in this study is in the range of caffeine contents of export standard Ethiopian coffee samples. Further study is required in which coffee samples could be analyzed with statistically sufficient data, coffee variety, agricultural and environmental factors as well as other components of coffee beans such as chlorogenic acid, trigonelline and sucrose in coffee beans.

Acknowledgements

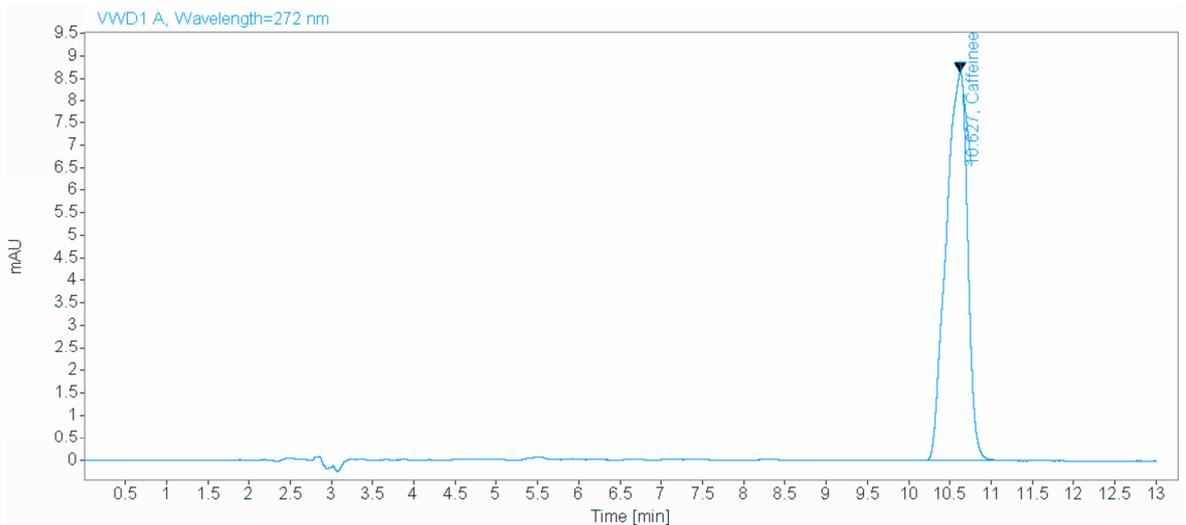
The authors primarily offer their deepest heart-felt thanks and glory to Almighty GOD. The authors are also acknowledge Wollega University and Ministry of Science and Higher Education for fellowships and financial support.

Appendix B: Chromatogram of 4 mg/L of Standard Caffeine**Appendix C: Chromatogram of 8 mg/L of Standard Caffeine****Appendix D: Chromatogram of 16 mg/L of Standard Caffeine**

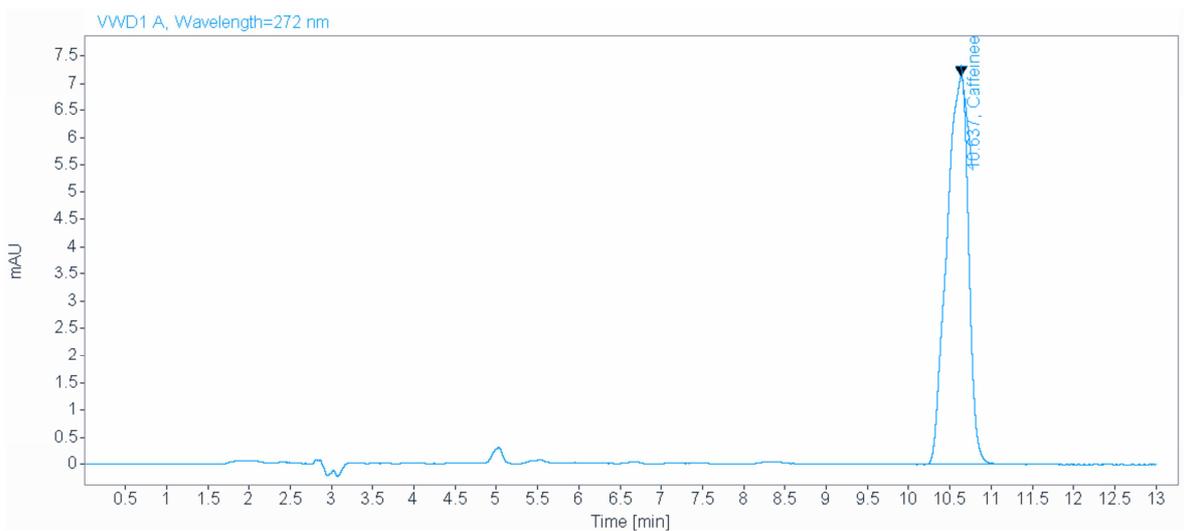
Appendix E: Chromatogram of Caffeine of Abe Dongoro Coffee Sample

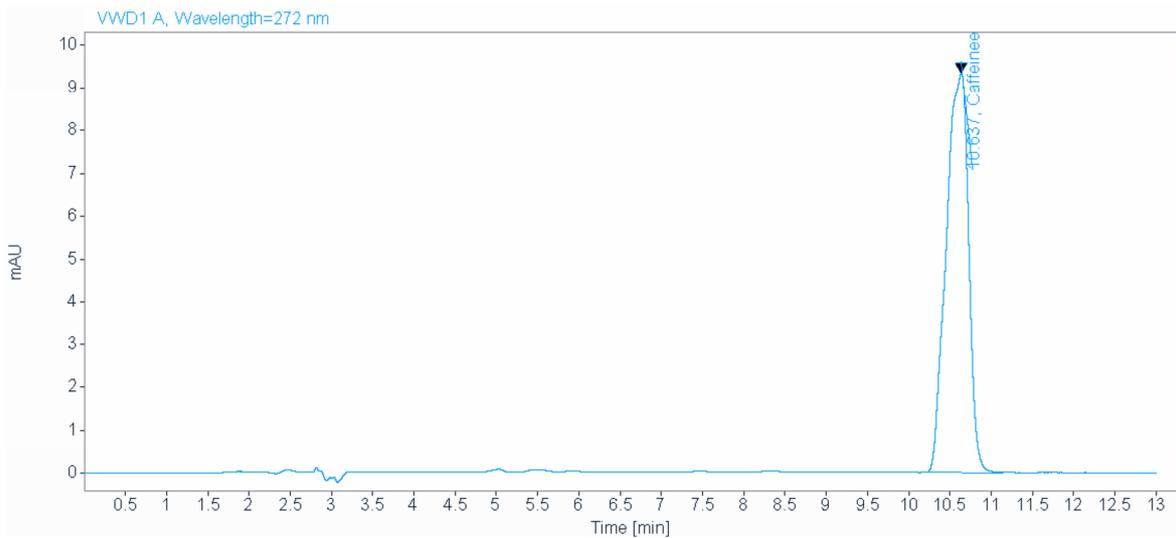
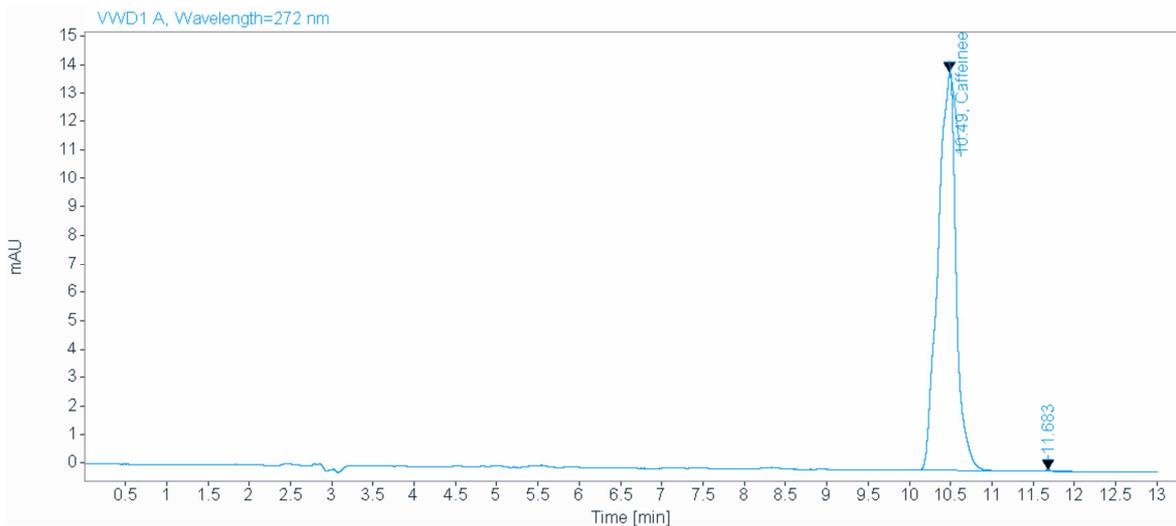
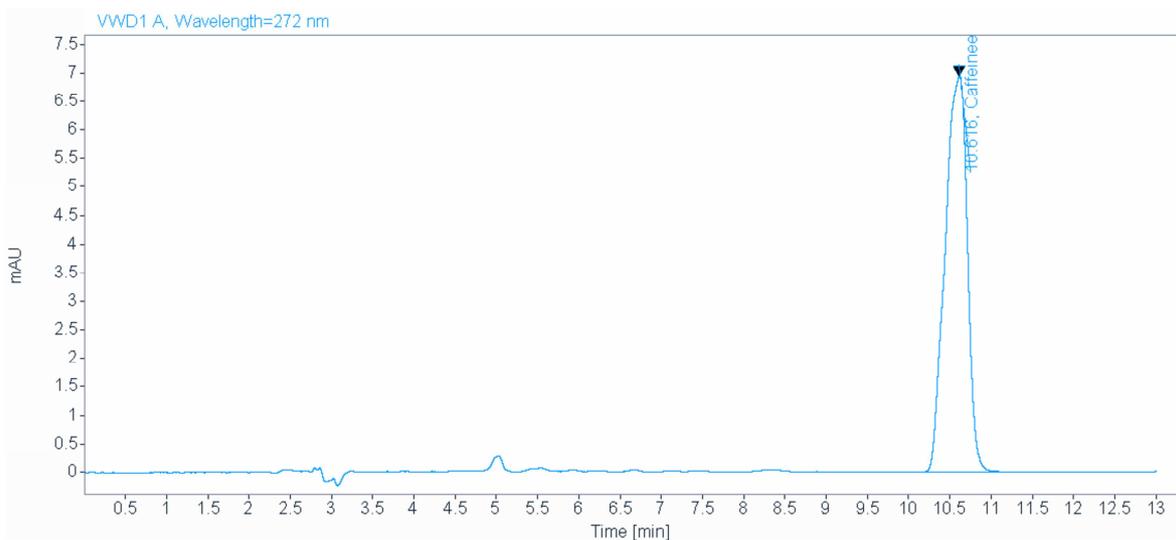


Appendix F: Chromatogram of Caffeine of Gida Ayana Coffee Sample



Appendix G: Chromatogram of Caffeine of Sasiga Coffee Sample



Appendix H: Chromatogram of Caffeine of Sibu Sire Coffee Sample**Appendix I: Chromatogram of Caffeine of Gida Ayana Coffee Sample (Spiked)****Appendix J: Chromatogram of Caffeine of Sasiga Coffee Sample (Duplicated)**

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