

Research Article

Simultaneous Determination of Caffeine and Chlorogenic Acids in Green Coffee by UV/Vis Spectroscopy

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Received 10 May 2017; Accepted 25 September 2017; Published 26 October 2017

Academic Editor: Maria B. P. P. Oliveira

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A simple method for the simultaneous determination of caffeine and chlorogenic acids content in green coffee was reported. The method was based on the use of UV/Vis absorption. It is relevant that the quantification of both caffeine and chlorogenic acids was performed without their preliminary chemical separation despite their spectral overlap in the range 250–350 nm. Green coffee was extracted with 70% ethanol aqueous solution; then the solution was analyzed by spectroscopy. Quantitative determination was obtained analytically through deconvolution of the absorption spectrum and by applying the Lambert-Beer law. The bands used for the deconvolution were the absorption bands of both caffeine and chlorogenic acids standards. The molar extinction coefficients for caffeine and chlorogenic acid in ethanol solution at 70% were calculated by using the chemical standards; the estimated values were $\epsilon_{(272\text{ nm})} = 12159 \pm 97 \text{ M}^{-1} \text{ cm}^{-1}$ for caffeine and $\epsilon_{(330\text{ nm})} = 27025 \pm 190 \text{ M}^{-1} \text{ cm}^{-1}$ for chlorogenic acids molecules, respectively. The estimate of concentration values was in agreement with the one obtained by High Performance Liquid Chromatography quantification. The method is fast and simple and allows us to realize routine controls during the coffee production. In addition, it could be applied on roasted coffee and espresso coffee.

1. Introduction

Coffee is an extremely popular beverage daily consumed. It has been the most commercialized food product and the most widely consumed beverage in the world. It is noteworthy that today coffee is considered as a functional food, primarily due to its high content of compounds that exert invigorating and antioxidant actions. These salutary effects are due mainly and, respectively, to caffeine and chlorogenic acids [1, 2]. Chlorogenic acids (CGAs) comprise a major class of phenolic compounds. The most abundant are the caffeoylquinic acids [3]. They account for approximately 80% of the total chlorogenic acids content [4]. The amount daily consumed of caffeine or CGAs is an important parameter to determine positive or negative effects on the health. Dose-response varies, partially reflecting genetic variation in susceptibility and consequently the different tolerance due to increasing exposure [5]. Thus, due to the increasing heed of consumer to safe food and to best final quality of the coffee, there is

a growing interest in the monitoring and quantification of caffeine and CGAs in coffee.

Caffeine is a heat stable bitter crystalline xanthine. It is an alkaloid acting as a mild psychoactive stimulant drug on the central nervous system. It is used both recreationally and medically in order to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs [6, 7]. It also possesses a weak diuretic action [8]. However, when excessively assumed, caffeine can cause unpleasant symptoms as well as state of excitement and anxiety. Finally, recommended daily intake could change depending on the health state of the consumer. The caffeine concentration depends on the coffee family: in *Coffea canephora var. robusta* it is approximately twice than in *Coffea arabica* [2].

Chlorogenic acids (CGAs) are classified according to the nature and number of cinnamic substituents and to the esterification position in the cyclohexane ring of the quinic acid [9]. 5-Caffeoylquinic acid is the most widely occurring and most studied CGAs. CGAs have antioxidant properties,

which play an important role in protecting food, cells, and any organ from oxidative degeneration [10] and also the coffee plant against microorganisms, insects, and UV radiation [11].

Moreover, CGAs contribute to prevent various diseases associated with oxidative stress, as well as cancer, aging, and cardiovascular and neurodegenerative diseases; besides, these substances have promising effects in modulating lipid and glucose metabolism [12–15]. On the other hand, quantity of CGAs is inversely associated with quality of a coffee cup. Indeed, their high amount in green coffee may produce undesirable flavor, possibly due to oxidation and degradation products formed before roasting [16] and to formation of phenols and catechols after roasting [17]. In addition, CGAs confer astringency, bitterness, and acidity to the coffee brew. The content of CGAs in *Coffea canephora* and *Coffea arabica* species varies considerably. In particular, it is about 4.0–8.0% in *Coffea arabica* and 6.0–11.5% in *Coffea canephora* var. *robusta* [11].

The conception of a fast, simple, and not expensive method aiming to the determination of both caffeine and chlorogenic acids into coffee may find high interest.

Several methods for the determination of caffeine in tea, coffee, and other beverages are available in literatures: UV/Vis spectrometry [7, 8, 18, 19], gas chromatography/mass spectrometry [20], High Performance Liquid Chromatography [21, 22], micellar electrokinetic chromatography [23], voltammetry [24], near-infrared spectroscopy [25], FT-Raman spectroscopy [26], and planar chromatography-multiple detection with confirmation by electrospray ionization mass spectrometry [27]. However, some of these methods need to use very expensive experimental techniques or chemical manipulation of the sample aiming to separate the substances to be analyzed. Anyhow, when separation does not occur, the use of very specialistic chemometric analysis methods proves to be indispensable.

The aim of this study was focused on the development of UV/Vis spectroscopy procedure, allowing us to determine at the same time the content of caffeine and CGAs without their mutual separation before the UV/Vis absorption measurements and when knowing the concentration of CGAs specific isomers is not needed. This method presents various advantages: it is sensitive, cheap, and easy regarding both the measurement procedure and the data analysis. The proposed protocol may allow replacing the use of expensive equipment, such as those required to apply standard High Performance Liquid Chromatography (HPLC) methods that at the moment limit the application in small industrial laboratories where a few analyses are performed each day [28–30]. The method here described could be applied to different states of coffee, such as beans, grounds coffee, coffee espresso, or brew, and to different food matrices having caffeine or CGAs as ingredients.

2. Materials and Methods

Green coffee samples were supplied by Angelo Morettino coffee company. They were five single-origin coffees, among which are three belonging to *Coffea arabica* and two to *Coffea canephora* var. *robusta* coffee. Caffeine ($C_8H_{10}N_4O_2$)

and chlorogenic acid (3-(3,4-dihydroxycinnamoyl) quinic acid, $C_{16}H_{18}O_9$) (CGA) chemical standards were bought from Sigma Aldrich (C0750-5G and C3878-1G, resp.). IUPAC numbering was used in the text. The ethanol solution at 70% (70% EtOH) was prepared using ethanol by Merck and bidistilled water.

2.1. Extracts Preparation. Several extraction protocols were employed aiming to determine the more appropriate experimental procedure to quantify caffeine and CGAs in coffee with a simple spectroscopic analysis. Particularly, extraction procedures were carried out, at both room and higher temperatures using water and/or ethanol as solvent. The more efficient, rapid, not invasive, and simple method was obtained by extraction with 70% EtOH at room temperature. The procedure was applied on both whole and ground coffee beans. The advantage of this protocol is the potential applicability in many coffee companies even if these are devoid of specific laboratory accessories or expensive equipment. For every monoorigin coffee, 3 g whole beans or 2 g ground coffee was, respectively, put in 60 ml or 100 ml of 70% EtOH at 25°C in the dark under magnetic stirring. After 24 h, the suspension was drawn by using a vacuum filtration system with a 0.20 μm filter (Sartolab BT 1000 system filter) to get rid of the suspended particles from the solution. After filtration, the sample was dissolved again with an equivalent volume of 70% EtOH for additional 24 h, in order to establish by second sample taking if the caffeine and CGAs were totally extracted in the first 24 h. The extract solutions were appropriately diluted in 70% EtOH in order to perform experimental measurements. Particularly, the extract solutions by whole beans were diluted 1:100; those derived by ground coffees were diluted 1:200 or 1:400 depending on the spectral intensity. The solutions derived from the different samples of Arabica coffees were named A1, A2, and A3. Analogously, the two different Robusta samples were named R1 and R2.

2.2. Standard Solution Preparation. For the standard solution preparation, commercial chemical standards of caffeine and CGA were used and dissolved in 70% EtOH previously filtered by using a 0.20 μm filter. Specifically, in order to minimize errors in the preparation procedure, both the chemical standards were first dissolved in alcoholic solution at 10 mg/ml and then, solutions at specific concentration, ranging from $1.0 \cdot 10^{-4}$ to $1.0 \cdot 10^{-2}$ mg/ml, were prepared by further dilution. The samples were uniformly dissolved by using magnetic stirrer for about 30 minutes. Then, UV/Vis absorbance spectra were recorded to determine the molar extinction coefficient values for the caffeine and CGA in 70% EtOH, respectively, at 272 and 330 nm, as well as expected when ethanol was mainly used as solvent [29]. The concentration values for caffeine and CGA were appropriately chosen to apply the Lambert-Beer law in the linear form.

2.3. UV/Vis Absorption Spectroscopy. UV/Vis absorption measurements were carried out on UV-2401PC Shimadzu spectrophotometer in the range 200–600 nm, by using halogen and deuterium sources for the visible and ultraviolet radiations, respectively. After the basic preparation, described

in Sections 2.1 and 2.2, the samples were appropriately diluted in 70% EtOH in order to make spectroscopic measurements. The contribution of the solvent absorption was subtracted from the obtained raw spectra. All measurements were made in triplicate at the controlled temperature of 25°C using a quartz square cuvettes with 1 cm optical path.

The determination of the molar extinction coefficients of caffeine at 272 nm and of CGA at 330 nm was realized by applying the well-known Lambert-Beer law, by fitting linearly the dependence of the absorbance versus the sample concentration. The error associated with the concentration was the standard deviation obtained from triplicate measurements.

By using Origin 8 and Excel 2013, spectra analysis was carried out. The Root Mean Squared Deviation (RMSD) between the acquired spectrum and the calculated profile verified the absorption profiles fittings. The RMSD value was for every fit lower than 10^{-4} .

2.4. HPLC Analysis. Chromatographic measurements were performed with a Shimadzu HPLC system (LC-2010 AT Prominence) equipped with a UV/Vis photodiode array detector (SPD-M20A), an on-line degasser system (DGU 20A5), and a 20 μ L sample loop. A C18 column (Kinetex 5U 100A size 250 \times 4.6 mm), maintained at 25°C by an oven (Knauer Jetstream Series), was used to separate caffeine from the chlorogenic acids, in order to quantify them. All the chromatographic analyses were performed by using as solvents trifluoroacetic acid 0.1% (v/v) in water (solvent A), and acetonitrile (solvent B). The mobile phase was pumped at 1.5 mL min⁻¹ using the following gradient profile: from 95% A and 5% B to 80% A and 20% B in 20 min followed by 10 min of isocratic and returning to initial condition at 35 min.

Calibration curves for both caffeine and 5-caffeoylquinic acid standards were obtained by injecting increasing concentrations of mixed standards and by plotting the peak area measured at wavelength 272 or 326 nm for caffeine and 5-caffeoylquinic acid, respectively, as function of their concentrations (Figures 3(a) and 3(b) right). The measurements were performed in triplicate. The complete spectrum from 200 to 600 nm was acquired and the appropriate wavelength was extracted to create the chromatographic profiles (272 nm and 326 nm, the wavelengths of maximum absorption for the caffeine and the CGA absorbance, resp.). The slope obtained from the linear fit for each calibration curve was used to estimate the concentration in all samples (A1, A2, A3, R1, and R2). The error associated with the concentration value was the standard deviation obtained from triplicate measurements.

2.5. Statistical Analysis. Data statistical analysis was performed using the STATSOFT 6.0 program (Vigonza, Padova, Italy).

The significant differences ($p \leq 0.05$) were evaluated by variance analysis [31], and the means separation was conducted using the Tukey post hoc test.

3. Results and Discussion

3.1. Chemical Standards Characterization. Figure 1 shows the UV/Vis absorption spectra of caffeine and CGA in 70% EtOH

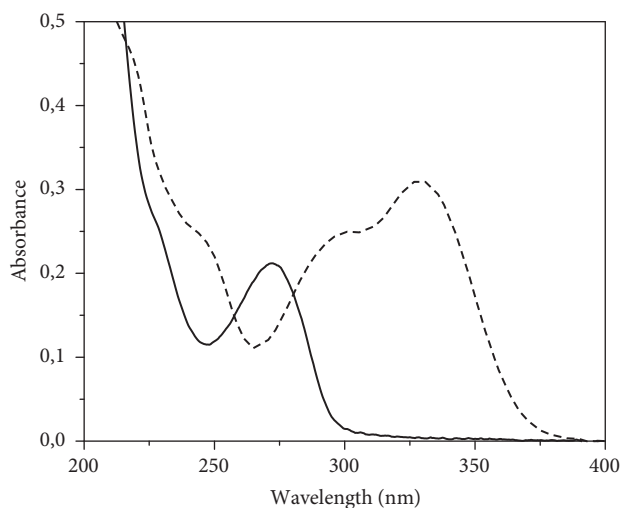


FIGURE 1: UV/Vis absorption spectra of caffeine (continuous line) and CGA (dashed line) chemical standard, respectively, at $5.3 \cdot 10^{-3}$ mg/ml and $8.5 \cdot 10^{-3}$ mg/ml. Measurements were performed at 25°C.

separately acquired in the spectral range 200–400 nm at 25°C. The caffeine spectrum was characterized by absorbance in the region 240–300 nm. The position of the maximum intensity was highly dependent on the used solvent. This band, centered at about 275 nm, was related to the C=O chromophore in caffeine molecule [32]. CGA absorbance was characterized by two maximum values, whose positions were once again dependent on the solvent. When ethanol was the prevalent solvent, the first band was localized at about 217 nm with a shoulder at about 240 nm, while the second band was at about 330 nm with a shoulder at 298 nm [29]. The band at about 330 nm was the highest peak and revealed the occurrence of a HOMO/LUMO transition with $\pi \rightarrow \pi^*$ character [33].

Caffeine spectra, as well as CGA spectra, were separately realized at different dilutions. The molar extinction coefficient values, at 272 nm for caffeine and at 330 nm for CGA, respectively, were calculated by fitting the linear dependence of absorbance amplitude by the sample concentration values. The resulting graphs were reported in Figures 2(a) and 2(b). The obtained values were $\epsilon_{(272\text{ nm})} = 12159 \pm 97 \text{ M}^{-1} \text{ cm}^{-1}$ for the caffeine and $\epsilon_{(330\text{ nm})} = 27025 \pm 190 \text{ M}^{-1} \text{ cm}^{-1}$ for CGA. These values were found in good agreement with values early obtained using different solvents and reported in the literature [34–36].

Calibration curves for chromatographic determination were obtained by triplicate HPLC measurements of caffeine and CGA solutions mixtures. Each calibration curve was obtained by injecting increasing concentrations of the mixed samples (in the same range of above UV/Vis measurements) and using the simple equation:

$$A = mC, \quad (1)$$

where A was the peak area calculated by HPLC software and C was the concentration of injected sample. The calibration curves estimated for caffeine and CGA by HPLC

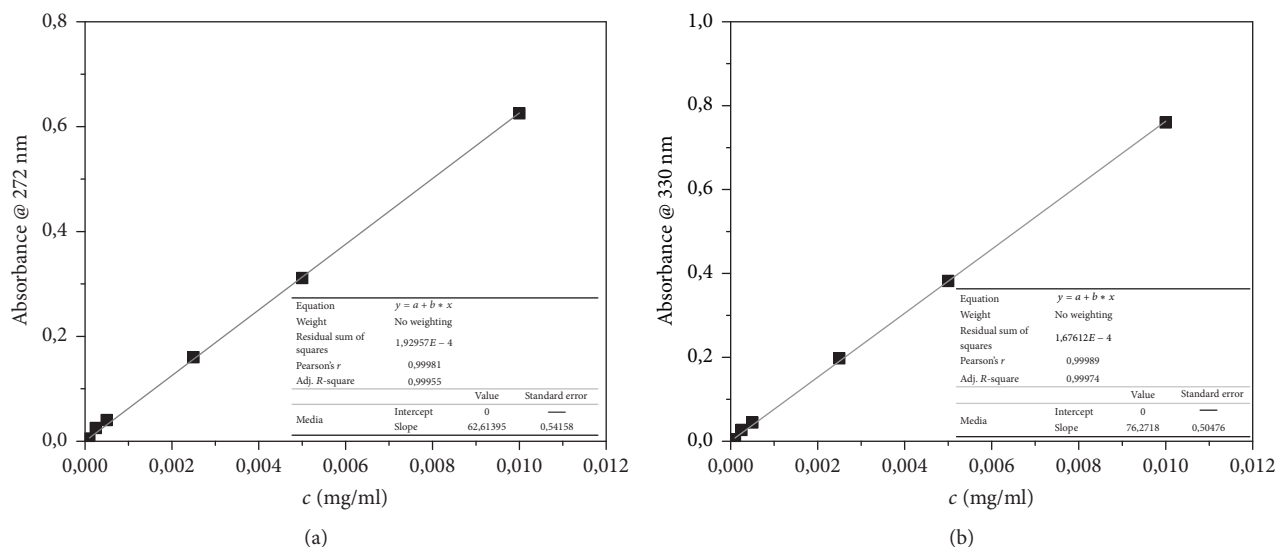


FIGURE 2: Absorbance values of caffeine (a) and CGA (b) as a function of the solution concentrations. The data correlation was calculated by a linear fit. The tables in the graph report the obtained parameters.

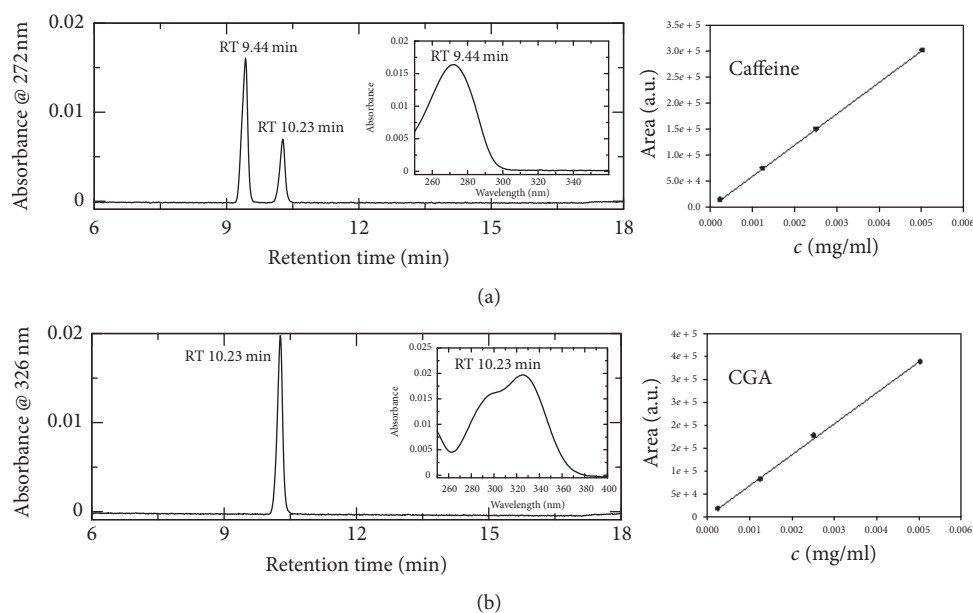


FIGURE 3: Chromatographic analysis for caffeine (a) and CGA (b). In the left the profiles of elution registered at the maximum of absorbance for caffeine or CGA as function of the retention time. The inset shows the spectrum at the maximum of peak. In the right, the calibration curves obtained by plotting the peak area as function of the concentration of injected sample. The lines represent the linear fit.

measurements were reported on the right of Figures 3(a) and 3(b) (see Section 2.4 for details). On the left of Figure 3, the chromatograms registered for the injection of the more diluted mixture were reported at two wavelengths: 272 and 326 nm, the wavelengths of maximum absorption for the caffeine and the CGA absorbance, respectively. The blue shift from 330 nm to 326 nm was ascribable to use of a different solvent as mobile phase. The peak at 9.44 min (Figure 3(a)) was corresponding to caffeine, as confirmed by the spectrum in the inset acquired at this elution time. The peak at 10.23 min (Figure 3(b)) was corresponding to CGA, as supported in

the inset. In Figure 3(a), CGA absorption was evident also at 272 nm due to its spectroscopic properties.

3.2. *Determination of the Caffeine and Chlorogenic Acids in Coffee.* Figure 4 shows the UV/Vis absorption spectra of the several extracts from ground green coffee acquired at 25°C in the 220–400 nm spectral range. All samples were characterized by an absorbance profile mainly resulting in the overlapping of caffeine and chlorogenic acids light absorption. The optical activity in this spectral range could be associated with $n \rightarrow \pi^*$ electronic transition of caffeine,

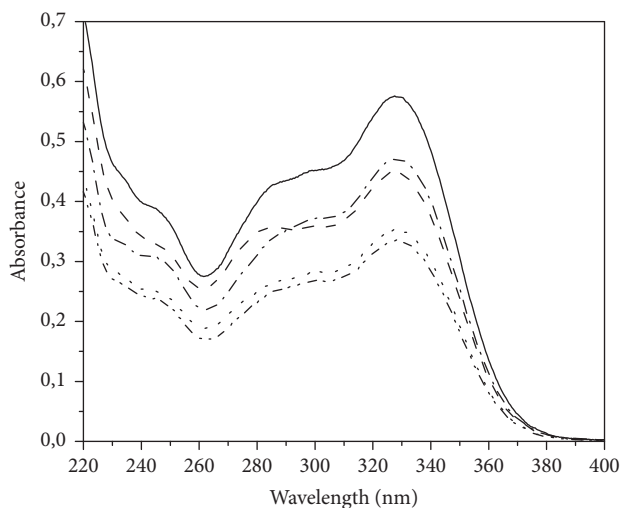


FIGURE 4: UV/Vis absorption spectra of A1 (dash dot), A2 (dash dot dot), A3 (dot), R1 (continuous), and R2 (dash) coffee chemical extraction.

chlorogenic acids, and trigonelline molecules [18]. Usually, the spectral interference between the caffeine absorbance and other UV absorption signals due to the other substances in the sample had been dissuaded from the use of UV/Vis spectroscopy to obtain a correct determination of caffeine or chlorogenic acids content in coffee [30, 37]. However, some methods aiming to use this technique were proposed [32, 35], but they need an early chemical separation of the substances having an absorbance in the same spectral range or a specialist chemometric analysis of the data. The method here described allows minimizing the sample treatment and obtaining simultaneously the quantification of caffeine and chlorogenic acids in coffee, realizing a spectral separation of their absorption contributions rather than an actual chemical separation. The spectral separation was obtained by analytical elaboration of the caffeine and CGA standards spectra. The spectra, already reported in Figure 1, were appropriately summed applying the formula

$$\text{Abs}_{\text{tot}}(\nu) = k_1 \text{Caff}(\nu) + k_2 \text{CGA}(\nu), \quad (2)$$

where $\text{Caff}(\nu)$ and $\text{CGA}(\nu)$ were the absorbance values at the change of the wavelength ν for caffeine and CGA molecules and k_1 and k_2 were two constant values. The k values were appropriately calculated by fitting a convolution of the absorption spectrum of the caffeine and CGA standards, in order to obtain the total absorption profile that better overlapped the spectral profile of green coffee spectrum in the spectral region 260–400 nm. The good overlap was verified by calculating the Root Mean Squared Deviation (RMSD) considering the original profile of the absorption spectrum of the coffee extract as the expected value and the calculated profile from the convolution of the standard's absorption bands as the obtained value. The RMSD value was for every fit lower than 10^{-4} . Figure 5 shows the analysis for A2 sample, as an example. Similar overlap was also obtained for other samples. This method has allowed verifying that mainly

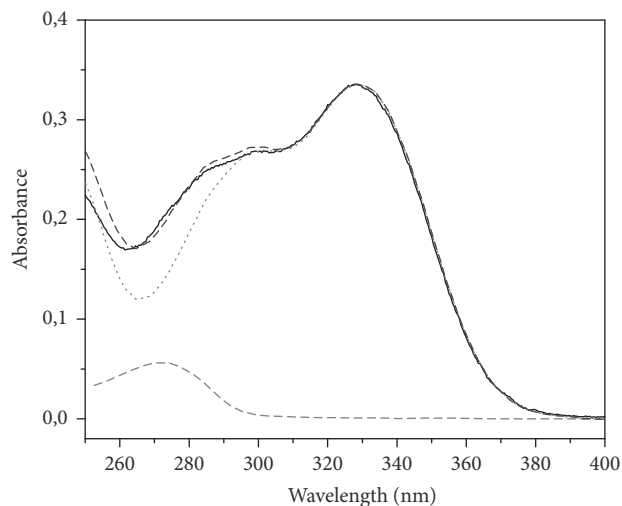


FIGURE 5: UV/Vis absorption spectra of coffee chemical extraction for R2 sample (black continuous line), of caffeine after multiplication for k_1 (grey dashed line), and of CGA after multiplication for k_2 (grey dotted line). In black dashed line the spectral overlap of the caffeine and CGA is reported.

caffeine and chlorogenic acids contributed to the absorption profile in the spectral range 260–400 nm. Moreover, the method has allowed the determination of the absorbance contributions due to the caffeine and CGA and consequently the determination of their concentration in coffee. The error associated with the concentration value was the standard deviation obtained from triplicate measurements. The values obtained, reported in Table 1, are in agreement with expected values depending on the type of the different species of coffees analyzed.

In order to test the above described procedure to determine caffeine and chlorogenic acid concentrations, all the green coffee ethanolic extracts were analyzed by HPLC and the estimate of concentrations was obtained by using the calculated calibration curves. As an example, the elution profiles for A2 sample were presented in Figure 6, where the peak at 9.44 min (a) showed the elution of caffeine, as previously reported for the caffeine standard. The concentration was estimated by applying (1) and using the m value estimated for caffeine and the peak area A calculated by HPLC software. The peak at 10.23 min in Figure 6(b) corresponded to CGA. Moreover, other substances were detected at 326 nm. The relative absorption spectra were extracted at the retention time and were reported in the inset of Figure 6(b). The comparison showed a similarity with the absorption profile of CGA standard used. We speculated that these peaks, corresponding to other chlorogenic acids present at low concentration in green coffee, were separated by liquid chromatography due to the difference in the chemical composition. However, the contribution of these molecules was surely embedded into the optical absorption spectrum registered for the samples by UV/Vis measurements. Moreover, the similarity of their absorption profiles to that of CGA standard assured that their contribution in the concentration calculation was anyway considered by having selected an appropriate value of k_2

TABLE 1: Percent quantification of caffeine and CGAs into ground coffee of Arabica (A1, A2, and A3) and Robusta (R1 and R2) coffee as obtained by applying UV/Vis absorption and HPLC methods. Error associated with calculated concentration value was the standard deviation obtained from triplicate measurements.

Samples	% caffeine		% CGAs	
	UV/Vis determination	HPLC determination	UV/Vis determination	HPLC determination
A1	$0,94 \pm 0,20^{ns}$	$1,19 \pm 0,25^{ns}$	$6,17 \pm 0,75^{ns}$	$7,01 \pm 0,85^{ns}$
A2	$0,90 \pm 0,05^{ns}$	$0,95 \pm 0,05^{ns}$	$4,40 \pm 0,50^{ns}$	$4,96 \pm 0,57^{ns}$
A3	$1,09 \pm 0,31^{ns}$	$0,85 \pm 0,24^{ns}$	$5,89 \pm 0,25^{ns}$	$5,65 \pm 0,24^{ns}$
R1	$3,01 \pm 0,53^{ns}$	$3,65 \pm 0,64^{ns}$	$15,05 \pm 0,09^{ns}$	$15,15 \pm 0,09^{ns}$
R2	$1,66 \pm 0,19^{ns}$	$1,87 \pm 0,21^{ns}$	$5,89 \pm 0,28^{ns}$	$5,64 \pm 0,26^{ns}$

ns: no significant difference (ANOVA).

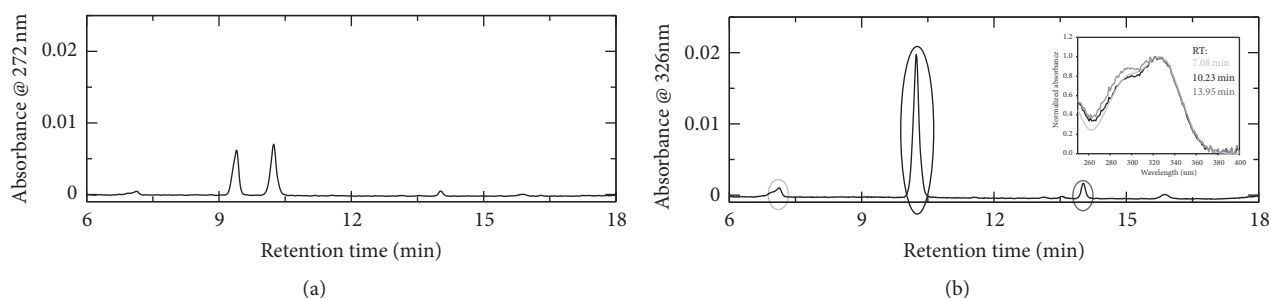


FIGURE 6: Chromatographic profiles registered at the maximum of absorbance for caffeine (a) and CGAs (b) as function of the retention time. The inset in (b) shows the normalized spectra for species evidenced in the plot.

factor in the formula (2). So, the method based on the use of UV/Vis spectroscopy allowed the determination of the concentration of the chlorogenic acids, although only CGA standard was used.

Consequently, the chlorogenic acids (CGAs) concentration determined by HPLC was calculated by considering the relative contribution of all the peaks. So, to calculate the concentration relative to CGAs, the sum of total area was used in formula (1) together with the m value estimated for CGA. The results obtained by UV/Vis elaboration and HPLC were reported and compared in Table 1. The data were not different according to ANOVA statistical analysis. The data reported in Table 1 referred to investigation carried out on the ground coffee samples. Indeed, when whole beans were taken into consideration, caffeine and CGAs were not efficiently extracted in the first 24 hours. However, it is relevant to underline that, also in that case, the caffeine and CGAs quantifications obtained by the UV/Vis spectrometric method were in agreement with the findings derived by using HPLC technique.

Summarizing, the findings show that the protocol used allows determining the concentration of caffeine and CGAs in coffee when knowing the concentration of specific isomers of the last is not needed. The calculation consists in convoluting the absorption spectra for caffeine and CGA standards in the spectral range 250–400 nm. This approach, for the first time at our knowledge, allows determining the concentrations of caffeine and CGAs simultaneously by using UV/Vis absorption spectroscopy without a preliminary chemical separation. On the base of the obtained results and the acquired knowledge, a very fast method may be designed.

Indeed, the separation of caffeine and CGAs by the analysis of the spectrum profile of the green coffee extract here performed allows determining the CGAs concentration by the application of the Lambert-Beer law using the absorbance in the range where caffeine is not absorbed. Then, caffeine concentration may be calculated from the difference of the absorbance value of the coffee extract and of the CGAs at the selected wavelength, for example, 270 nm. The method for the determination of caffeine and CGAs amount in coffee may be simply and quickly applied after the design of a spectrophotometric device that, based on a simple analysis of the spectrum reading, gives back the caffeine and chlorogenic acids concentration values. The method here proposed embeds an additional advantage: it is based on the use of an UV/Vis spectrophotometer, being easy, fast, and cheap device, today available in most laboratories. Moreover, its application makes the increase of knowledge more simple on the product in coffee makers and in the consumers also when this is not required by law as in the case of the decaffeinated coffee.

4. Conclusions

A specific procedure for the quantification of caffeine and chlorogenic acids in coffee was reported. The goodness of the results was verified by comparing them with those derived by using a conventional method of quantification of caffeine and CGAs, as the HPLC analysis. The proposed method embeds several advantages: it makes use of an UV/Vis spectrophotometer being easy, fast, and cheap device, today available in most laboratories. For the first time at

our knowledge, it allows the simultaneous determination of the caffeine and CGAs content without the necessity to separate them chemically beforehand. This analytical method may therefore be recommended for the rapid and corrected quantification of caffeine and CGAs in coffee. It may make the increase of knowledge more simple on the product in coffee makers and in consumers also when this is not required by law as in the case of the decaffeinated coffee.

Moreover, the method may be also applied on roasted coffee, espresso, and other beverages containing caffeine and/or chlorogenic acids.

Abbreviations

HPLC: High Performance Liquid Chromatography

CGAs: Chlorogenic acids

CGA: 3-(3,4-Dihydroxycinnamoyl) quinic acid.

Conflicts of Interest

All the authors declare to have no potential conflicts of interest. The authors have no relationship with the organization that funded the research.

Authors' Contributions

G. Navarra and M. Moschetti contributed equally to this work and should be considered co-first authors.

Acknowledgments

The authors wish to thank Dr. Margherita Amenta for her useful support. Special thanks are due to Dr. Pier Luigi San Biagio for his precious support. This work was supported by the project CAFIS "Controllo della Qualità e della Salubrità nella Filiera del Caffè con Tecniche Chimico-Fisiche Tipiche della Scienza dei Materiali" (P. O. R. Sicilia Misura 4.1.1.Ibis, FESR 2007–2013, CUP G73F12000120004).

References

- [1] Y.-F. Chu, *Coffee: emerging health effects and disease prevention*, John Wiley and Sons, 2012.
- [2] T. W. M. Crozier, A. Stalmach, M. E. J. Lean, and A. Crozier, "Espresso coffees, caffeine and chlorogenic acid intake: potential health implications," *Food & Function*, vol. 3, no. 1, pp. 30–33, 2012.
- [3] M. N. Clifford, K. L. Johnston, S. Knight, and N. Kuhnert, "Hierarchical scheme for LC-MSⁿ identification of chlorogenic acids," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 10, pp. 2900–2911, 2003.
- [4] A. Farah, T. de Paulis, L. C. Trugo, and P. R. Martin, "Effect of roasting on the formation of chlorogenic acid lactones in coffee," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 5, pp. 1505–1513, 2005.
- [5] P. J. Rogers, C. Hohoff, S. V. Heatherley et al., "Association of the anxiogenic and alerting effects of caffeine with ADORA2A and ADORA1 polymorphisms and habitual level of caffeine consumption," *Neuropsychopharmacology*, vol. 35, no. 9, pp. 1973–1983, 2010.
- [6] A. Nehlig, J.-L. Daval, and G. Debry, "Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects," *Brain Research Reviews*, vol. 17, no. 2, pp. 139–170, 1992.
- [7] A. B. M. A. Maidon, A. O. Mansoer, and H. Sulistyarti, "Study of various solvents for caffeine determination using UV spectrophotometric," *Journal of Applied Sciences Research*, vol. 8, no. 5, pp. 2439–2442, 2012.
- [8] Amos-Tautua, W. B. Martin, and E. R. E. Diepreye, "Ultra-violet spectrophotometric determination of caffeine in soft and energy drinks available in Yenagoa, Nigeria," *Advance Journal of Food Science and Technology*, vol. 6, no. 2, pp. 155–158, 2014.
- [9] M. N. Clifford, "Chlorogenic acids and other cinnamates—nature, occurrence, dietary burden, absorption and metabolism," *Journal of the Science of Food and Agriculture*, vol. 80, no. 7, pp. 1033–1043, 2000.
- [10] D. P. Moreira, M. C. Monteiro, M. Ribeiro-Alves, C. M. Donangelo, and L. C. Trugo, "Contribution of chlorogenic acids to the iron-reducing activity of coffee beverages," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 5, pp. 1399–1402, 2005.
- [11] A. Farah and C. M. Donangelo, "Phenolic compounds in coffee," *Brazilian Journal of Plant Physiology*, vol. 18, no. 1, pp. 23–36, 2006.
- [12] B. K. Bassoli, P. Cassolla, G. R. Borba-Murad et al., "Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: Effects on hepatic glucose release and glycaemia," *Cell Biochemistry & Function*, vol. 26, no. 3, pp. 320–328, 2008.
- [13] K. Fujioka and T. Shibamoto, "Chlorogenic acid and caffeine contents in various commercial brewed coffees," *Food Chemistry*, vol. 106, no. 1, pp. 217–221, 2008.
- [14] K. Huang, X.-C. Liang, Y.-L. Zhong, W.-Y. He, and Z. Wang, "5-Caffeoylquinic acid decreases diet-induced obesity in rats by modulating PPAR α and LXR α transcription," *Journal of the Science of Food and Agriculture*, vol. 95, no. 9, pp. 1903–1910, 2015.
- [15] C. Manach, A. Scalbert, C. Morand, C. Rémésy, and L. Jiménez, "Polyphenols: food sources and bioavailability," *American Journal of Clinical Nutrition*, vol. 79, no. 5, pp. 727–747, 2004.
- [16] A. Farah, M. C. Monteiro, V. Calado, A. S. Franca, and L. C. Trugo, "Correlation between cup quality and chemical attributes of Brazilian coffee," *Food Chemistry*, vol. 98, no. 2, pp. 373–380, 2006.
- [17] L. C. Trugo, "Encyclopedia of Food Sciences and Nutrition," L. C. Trugo and P. M. Finglas, Eds., pp. 1498–1506, 2nd edition, 2003.
- [18] U. T. C. P. Souto, M. J. C. Pontes, E. C. Silva et al., "UV-Vis spectrometric classification of coffees by SPA-LDA," *Food Chemistry*, vol. 119, no. 1, pp. 368–371, 2010.
- [19] S. Ahmad Bhawani, S. S. Fong, and M. N. Mohamad Ibrahim, "Spectrophotometric Analysis of Caffeine," *International Journal of Analytical Chemistry*, vol. 2015, Article ID 170239, 2015.
- [20] K. Shrivastava and H.-F. Wu, "Rapid determination of caffeine in one drop of beverages and foods using drop-to-drop solvent microextraction with gas chromatography/mass spectrometry," *Journal of Chromatography A*, vol. 1170, no. 1–2, pp. 9–14, 2007.
- [21] C. A. B. De Maria, L. C. Trugo, R. F. A. Moreira, and M. Petracco, "Simultaneous determination of total chlorogenic acid, trigonelline and caffeine in green coffee samples by high

- performance gel filtration chromatography," *Food Chemistry*, vol. 52, no. 4, pp. 447–449, 1995.
- [22] P. D. Tzanavaras and D. G. Themelis, "Development and validation of a high-throughput high-performance liquid chromatographic assay for the determination of caffeine in food samples using a monolithic column," *Analytica Chimica Acta*, vol. 581, no. 1, pp. 89–94, 2007.
- [23] A. D. Meinhart, C. S. Bizzotto, C. A. Ballus et al., "Optimisation of a CE method for caffeine analysis in decaffeinated coffee," *Food Chemistry*, vol. 120, no. 4, pp. 1155–1161, 2010.
- [24] T. Alizadeh, M. R. Ganjali, M. Zare, and P. Norouzi, "Development of a voltammetric sensor based on a molecularly imprinted polymer (MIP) for caffeine measurement," *Electrochimica Acta*, vol. 55, no. 5, pp. 1568–1574, 2010.
- [25] M. Ito, T. Suzuki, S. Yada et al., "Development of a method for the determination of caffeine anhydrate in various designed intact tables by near-infrared spectroscopy: A comparison between reflectance and transmittance technique," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 47, no. 4-5, pp. 819–827, 2008.
- [26] S. Armenta, S. Garrigues, and M. De La Guardia, "Solid-phase FT-Raman determination of caffeine in energy drinks," *Analytica Chimica Acta*, vol. 547, no. 2, pp. 197–203, 2005.
- [27] M. Aranda and G. Morlock, "Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks by planar chromatography-multiple detection with confirmation by electrospray ionization mass spectrometry," *Journal of Chromatography A*, vol. 1131, no. 1-2, pp. 253–260, 2006.
- [28] G. Alpdogan, K. Karabina, and S. Sungur, "Derivative spectrophotometric determination of caffeine in some beverages," *Turkish Journal of Chemistry*, vol. 26, no. 2, pp. 295–302, 2002.
- [29] A. Belay and A. Gholap, "Determination of integrated absorption cross-section, oscillator strength and number density of caffeine in coffee beans by the integrated absorption coefficient technique," *African Journal of Pure and Applied Chemistry*, vol. 3, no. 11, pp. 34–240, 2009.
- [30] Q.-L. Zhang, H.-Z. Lian, W.-H. Wang, and H.-Y. Chen, "Separation of caffeine and theophylline in poly(dimethylsiloxane) microchannel electrophoresis with electrochemical detection," *Journal of Chromatography A*, vol. 1098, no. 1-2, pp. 172–176, 2005.
- [31] M. Číž, H. Čížová, P. Denev, M. Kratchanova, A. Slavov, and A. Lojek, "Different methods for control and comparison of the antioxidant properties of vegetables," *Food Control*, vol. 21, no. 4, pp. 518–523, 2010.
- [32] L. López-Martinez, P. L. López-de-Alba, R. García-Campos, and L. M. De León-Rodríguez, "Simultaneous determination of methylxanthines in coffees and teas by UV-Vis spectrophotometry and partial least squares," *Analytica chimica acta*, vol. 493, no. 1, pp. 83–94, 2003.
- [33] J.-P. Cornard, C. Lapouge, L. Dangleterre, and C. Allet-Bodelot, "Complexation of lead(II) by chlorogenic acid: Experimental and theoretical study," *The Journal of Physical Chemistry A*, vol. 112, no. 48, pp. 12475–12484, 2008.
- [34] S. Banerjee, P. K. Verma, R. K. Mitra, G. Basu, and S. K. Pal, "Probing the interior of self-assembled caffeine dimer at various temperatures," *Journal of Fluorescence*, vol. 22, no. 2, pp. 753–769, 2012.
- [35] A. Belay, K. Ture, M. Redi, and A. Asfaw, "Measurement of caffeine in coffee beans with UV/vis spectrometer," *Food Chemistry*, vol. 108, no. 1, pp. 310–315, 2008.
- [36] A. Cesaro, E. Russo, and V. Crescenzi, "Thermodynamics of caffeine aqueous solutions," *The Journal of Physical Chemistry*, vol. 80, no. 3, pp. 335–339, 1976.
- [37] P. Ortega-Barrales, R. Padilla-Weigand, and A. Molina-Díaz, "Simultaneous determination of paracetamol and caffeine by flow injection-solid phase spectrometry using C18 silica gel as a sensing support," *Analytical Sciences*, vol. 18, no. 11, pp. 1241–1246, 2002.

