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Simultaneous Spectrometric Determination of Caffeic Acid, Gallic Acid, and Quercetin in Some Aromatic Herbs, Using Chemometric Tools

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ABSTRACT. The purpose of this work is the development of a method for an effective, less expensive, rapid, and simultaneous determination of three phenolic compounds (caffeic acid, gallic acid, and quercetin) widely present in food resources and known for their antioxidant powers. The method relies on partial least squares (PLS) calibration of UV-visible spectroscopic data. This model was applied to simultaneously determine, the concentrations of caffeic acid (CA), gallic acid (GA), and quercetin (Q) in six herb infusion extracts: basil, chive, laurel, mint, parsley, and thyme. A wavelength range (250-400) nm, and an experimental calibration matrix with 21 samples of ternary mixtures composed of CA (6.0-21.0 mg/L), GA (10.0-35.2 mg/L), and Q (6.4-17.5 mg/L) were chosen. Spectroscopic data were mean-centered before calibration. Two latent variables were determined using the contiguous block cross-validation procedure after calculating the root mean square error cross-validation *RMSECV*. Other statistic parameters: *RMSEP*, R^2, and Recovery (%) were used to determine the predictive ability of the model. The results obtained demonstrated that UV-visible spectrometry and PLS regression were successfully applied to simultaneously quantify the three phenolic compounds in synthetic ternary mixtures. Moreover, the concentrations of CA, GA and Q in herb infusion extracts were easily predicted and found to be 3.918–18.055, 9.014–23.825, and 9.040–13.350 mg/g of dry sample, respectively.

Key words: Simultaneous determination, Chemometrics, Phenolic compounds, UV-visible, Herbs

INTRODUCTION

Polyphenols are a family of organic molecules widely present in the plant realm. They are characterized, as the name suggests, by the presence of several phenolic groups associated with more or less complex structures, generally of high molecular weight. These compounds are the products of the secondary metabolism of plants.[1] They can be classified into flavonoids and non-flavonoids.[2] Flavonoids include anthocyanins, tannins, flavonols, and flavanols, free or polymerized. Non-flavonoids include phenolic acids, cinamic acids, stilbenes, phenolic alcohols, and aldehydes.[3] These compounds may be responsible for the biological activity of the plant.[4,5]

Polyphenols have special importance due to their antioxidant properties and it is well known that phenolic compounds have positive effects on human health.[6] Thus, regular consumption of fruit and vegetables has been associated with reduced the risk of certain types of cancer and cardiovascular diseases.[7] Therefore, the identification, and determination of polyphenol compounds in food products become more important. These procedures generally require a prior separation to be performed, usually based on reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with UV-Vis detection and/or mass (LCMS) or tandem mass spectrometry (LCMS/MS).[8–13] These techniques take time to carry out, require expensive equipment, and the use of several solvents.[14] In addition, some of these methods involve fastidious safety measure, physical separation, first round treatment and sophisticated apparatus usually not easy to get to available in all laboratories.[15] To work around this problem, chemists use multivariate analysis methods called chemometrics. These methods allow the analysis of mixtures by spectroscopy without the need for chromatography thus, simplifying the analysis and reducing its time and cost. Many studies using spectroscopic analysis and chemometric methods have been performed. They gave very satisfactory results in qualitative and quantitative analysis of mineral and organic compounds. Out of these methods, the most widespread is the partial least squares (PLS) regression. The results of this method were used to relate the concentrations of the constituents of the mixtures to their spectral properties.[16–26] The theory of PLS method has been discussed by several authors and one well-recognized algorithm for computing PLS regression components is SIMPLS.[27–31]

The objective of this kind of research works is to achieve an alternative to the use of laborious techniques, which require expensive equipment, the use of many solvents and a long execution time. The aim of this work is to develop chemometric methods combined with a simple analysis method (UV-Vis) for the simultaneous determination of phenolic compounds present in synthetic and natural mixtures (aromatic herbs). In order to develop a method for an effective, less expensive, rapid, and simultaneous determination of three phenolic compounds (caffeic acid, gallic acid, and quercetin) widely present in food resources and known for their antioxidant powers. The method relies on partial least squares (PLS) calibration of UV-Visible spectroscopic data.

EXPERIMENTAL

Materials

Caffeic acid (>98%), gallic acid (>99%), quercetin (>95%) are Sigma-Aldrich reagents. All the other reagents used are of analytical grade and used without purification.

Ternary Mixtures of Standard Samples

Appropriate volumes of caffeic acid, gallic acid, and quercetin were transferred to 25 mL volumetric flasks. The solutions were thoroughly mixed and made up to the mark with doubly distilled water. The final concentrations of these solutions were kept in their linear determination ranges. All the samples were prepared in triplicate to minimize errors of measurements.

Herb Samples

The studied material consisted of some herbs commonly used in households: basil (Ocimum basilicum), chive (Allium schoenoprasum), laurel (Laurusnobilis), mint (Mentha piperita), parsley (Petroselinum crispum), and thyme (Thymbra spicata). These studied herbs were purchased directly from local markets in the north of Algeria. They included both imported and locally made products. All herbal samples were treated in an identical manner. The extracts of basil, chive, laurel, mint, parsley, and thyme, are obtained by infusion of 0.1 g of dry matter in 100 mL of doubly distilled water for 15 minutes, then filtered and cooled in the dark. For each herb, we used at least three Algerian brands and we considered and presented the average in the "results and discussion" section.

UV-visible Absorption Spectral Data and Chemometric Software

All absorbance measurements were collected on a Shimadzu UV2102PC double bean UV-Vis spectrophotometer, using a 10 mm quartz cell. The recorded spectra were digitized with an interval of 0.5 nm between consecutive points. Therefore, 301 data points were used to represent a spectrum in the range of (250–400) nm. For each measurement, about 2 mL of the above solution were transferred to a spectrophotometric cell and the spectra of all prepared solutions were recorded against the reagent blank.

Chemometric analysis was performed with Solo_MIA 8.5.2 software from Eigenvector Research, Inc., Manson, WA USA. Partial least squares regression (PLS) was applied on UV visible spectroscopic data. Before the calibration, all the spectral data were mean-centered.

RESULTS AND DISCUSSION

The spectra in *Fig.* 1 show that caffeic acid, gallic acid and quercetin are highly absorbing and highly overlapping in the range of (250–400) nm. Caffeic and Gallic acids were dissolved in doubly distilled water. The caffeic acid spectrum has two absorption maxima at 288 nm and 312 nm and the Gallic acid spectrum has maximum absorption at 267 nm. Quercetin was dissolved in ethanol; its spectrum has two absorption maxima at 256 nm and 373 nm.

Univariate Calibration

The calibration curves of caffeic acid, gallic acid and quercetin were calculated after reading the absorbance of the standard solutions, and corresponding equations were reported in *Table* 1. The linear ranges were 3–30 mg/L, 2–50 mg/L and 1.5–25 mg/L for caffeic acid, gallic acid and quercetin, respectively. *Table* 1 also presents the cal-

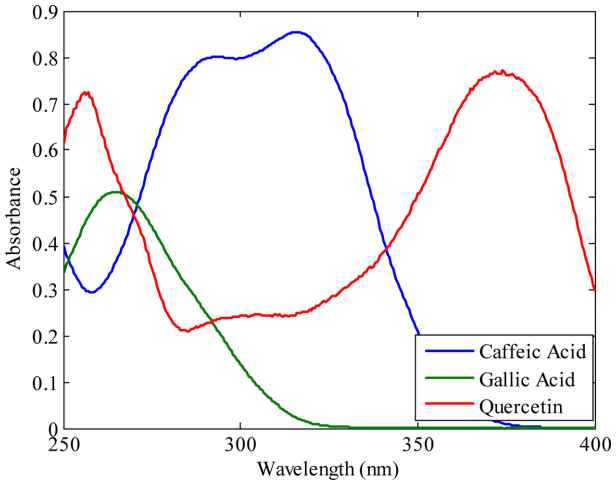

***Figure* 1.** Absorbance spectra of caffeic acid, gallic acid and quercetin. Concentration of each component is 10 mg/L.

***Table* 1.** Results of univariate calibration

Compound	λ_{max} (nm)	Linear range (mg/L)	Calibration equation	R^2
Caffeic acid	288	3 – 30	A = (0.077 ± 0.002[a]) C + (0.028 ± 0.002)	0.999 ± 0.019
	312	3 – 30	A = (0.083 ± 0.003) C + (0.005 ± 0.001)	0.999 ± 0.025
Gallic acid	267	2 – 50	A = (0.049 ± 0.002) C + (0.023 ± 0.002)	0.999 ± 0.025
Quercetin	256	1.5 – 25	A = (0.072 ± 0.002) C + (0.005 ± 0.001)	0.999 ± 0.026
	373	1.5 – 25	A = (0.077 ± 0.003) C + (0.002 ± 0.000)	0.999 ± 0.027

[a]Standard deviation

ibration equations at λ_{max} of each compound, linear range and determination coefficients.

Multivariate Calibration

Experimental Design. The design used for the calibration experiments maximizes the information content in the spectra. A training set of 21 samples, s1 to s21, was produced and arranged, as indicated in *Table* 2. In addition, seven further samples, s22 to s28, were chosen for a test set to span the calibration range. When working with three components (in this case caffeic acid, gallic acid, and quercetin), the experimental domain corresponds to a triangle. A point in this plane can identify all possible mixtures. In this work, a six concentration level design was chosen (a reduced 63 design).

The reduction was performed by choosing a subset of all possible 216 experiments with a number of properties.

***Table* 2.** Concentration data for calibration set mixtures of caffeic acid, gallic acid and quercetin

Calibration sample	Concentration (mg/L)		
	Caffeic acid	Gallic acid	Quercetin
S1	21	10.0	6.40
S2	18	10.0	8.62
S3	18	15.2	6.40
S4	15	10.0	10.84
S5	15	15.2	8.62
S6	15	20.0	6.40
S7	12	10.0	13.06
S8	12	15.2	10.84
S9	12	20.0	8.62
S10	12	25.2	6.40
S11	9	10.0	15.28
S12	9	15.2	13.06
S13	9	20.0	10.84
S14	9	25.2	8.62
S15	9	30.0	6.40
S16	6	10.0	17.50
S17	6	15.2	15.28
S18	6	20.0	13.06
S19	6	25.2	10.84
S20	6	30.0	8.62
S21	6	35.2	6.40

There are six mixtures for each compound at each concentration level. This design spans the mixture space fairly well. Each level corresponds to a concentration, the lowest level to the minimum, and the highest to the maximum.[32,33] The true concentrations of each compound at six concentration levels are given in *Table* 2.

Selection of Optimum Number of Factors. In this work, the cross-validation method was used to select the number of significant PLS components for modeling the system without over fitting the concentration data. The root mean square error cross-validation *RMSECV* obtained by optimizing the calibration matrix of the absorbance data with PLS was shown in *Fig.* 2. A good rule for choosing the number of PLS components to retain corresponds to the minimum of the *RMSECV*, so the optimum numbers of components obtained was 2.

The relation between samples (scores) in *Fig*. 3 shows a triangle formed with 21 calibration samples as explained above in the experimental design. The scores on latent variable 1 (61.52%) increase with the concentration of caffeic acid (sample s1 contains a maximum of caffeic acid and a

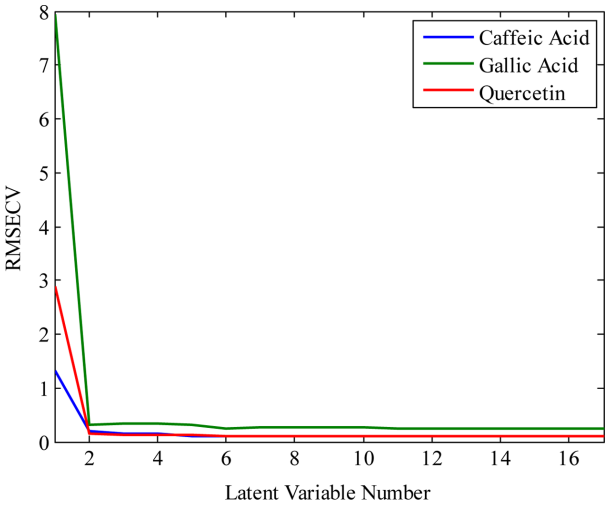

***Figure* 2.** Representation of the *RMSECV* vs. Latent Variable Number.

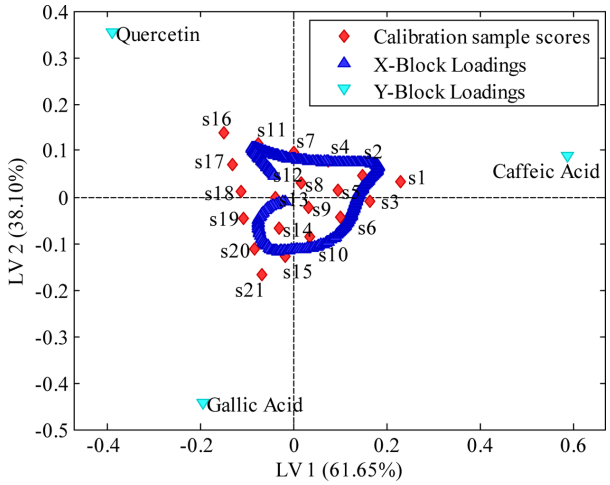

***Figure* 3.** Representation of bi-plot scores and loadings on (LV1, LV2) plan.

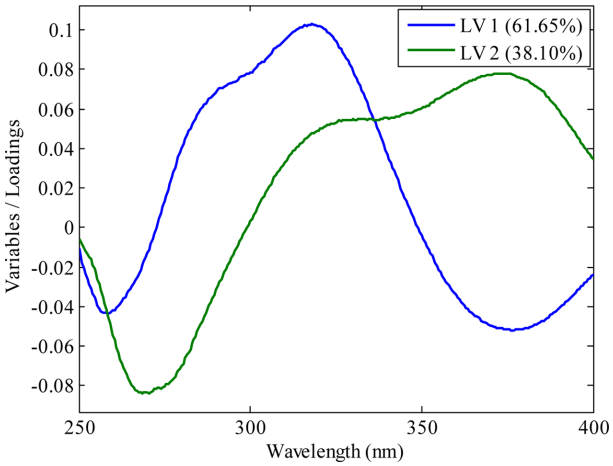

***Figure* 4.** Loadings on LV 1 and LV 2.

minimum of gallic acid and quercetin) while the scores on latent variable 2 (38.24%) increase with the concentration of quercetin (sample s16 contains a maximum of quercetin and minimums of caffeic and Gallic acids).

The relation between variables (loadings) in *Fig.* 4 shows optimums in (258 nm, 290 nm, 320 nm, and 375 nm) for LV1 and (270 nm, 323 nm, and 375 nm) for LV2.

We can also see similarities in some regions with the individual spectra of caffeic acid, gallic acid and quercetin shown in *Fig.* 1. The loadings on LV1 (61.65%) closely resembles the spectrum of caffeic acid in the range of (250 nm – 375 nm) and is opposite to the spectra of gallic acid (250 nm – 320 nm) and quercetin (250 nm – 400 nm). On the other hand, the loadings on LV2 (38.10%) closely resembles the spectrum of quercetin in the range of (320 nm – 400 nm) and is opposite to the spectrum of gallic acid in the range of (250 nm – 320 nm).

Statistical Parameters

It is useful to introduce several measures of the models fit to the data and predictive power. Two general statistical parameters: Root mean square error calibration (*RMSEC*) and Root mean square error cross validation (*RMSECV*) were selected to evaluate the calibration step of the model using 21 calibration samples. Three statistical parameters: Root mean square error prediction (*RMSEP*), *Recovery* (%), and determination coefficient (R^2) were selected to evaluate the prediction ability of the model for the simultaneous determination of caffeic acid (CA), gallic acid (GA), and quercetin (Q) in the test step using seven samples. The composition of the test samples on CA, GA, and Q, and the results obtained by applying PLS model to the seven test set samples are listed in *Table* 3. Recovery values are very close to 100% and this result verifies the good performance of the PLS model in predicting the concentrations of the phenolic compounds in ternary mixtures. Other statistical results are summarized in *Table* 4. The complexity of the model is very low (only two latent variables with 99.76% of variance), the values of *RMSEC*, *RMSECV*, and *RMSEP* are very close together, and the R^2 values are close to one. The results obtained allow us to confirm the successful applicability of the proposed model for the simultaneous determination of the three phenolic compounds tested.

***Table* 3.** Concentration data for prediction set mixtures and recovery values

Test sample	Composition (mg/L)	Recovery (%)		
		CA[a]	GA[b]	Q[c]
S22	CA(19), GA(11.73), Q(7.14)	98.97 ± 1.91[d]	103.09 ± 2.05	97.53 ± 1.74
S23	CA(15), GA(15.07), Q(8.62)	101.34 ± 1.56	97.37 ± 1.87	101.88 ± 1.62
S24	CA(12), GA(15.07), Q(10.84)	98.91 ± 1.33	98.79 ± 1.26	101.23 ± 1.59
S25	CA(10), GA(16.80), Q(11.58)	97.76 ± 1.28	101.32 ± 1.91	101.39 ± 1.60
S26	CA(9), GA(25.07), Q(8.62)	101.21 ± 1.30	98.36 ± 1.54	101.91 ± 2.11
S27	CA(6), GA(15.07), Q(15.28)	101.34 ± 1.39	101.44 ± 1.80	98.73 ± 1.14
S28	CA(6), GA(30.13), Q(8.62)	101.86 ± 1.42	101.27 ± 1.88	98.13 ± 1.37

[a]Caffeic acid; [b]Gallic acid; [c]Quercetin; [d]Standard deviation.

***Table* 4.** Statistical parameters using PLS model

Compound	Number of LVs	RMSEC	RMSECV	RMSEP	R^2
Caffeic A	2	0.179	0.203	0.206	0.999
Gallic A	2	0.261	0.305	0.302	0.999
Quercetin	2	0.137	0.154	0.159	0.999

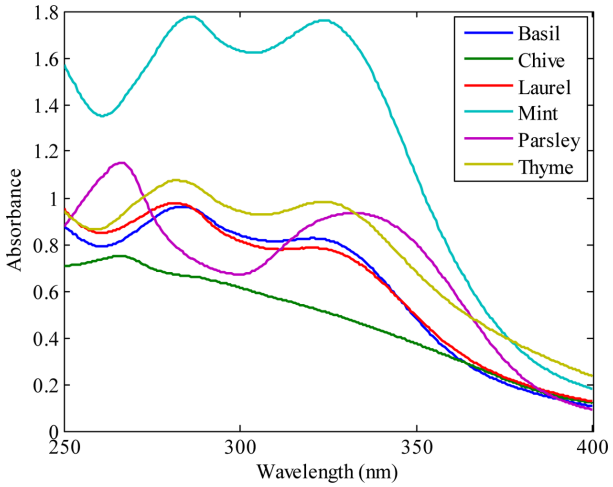

***Figure* 5.** Representation of the UV spectra of the six aqueous herb extracts.

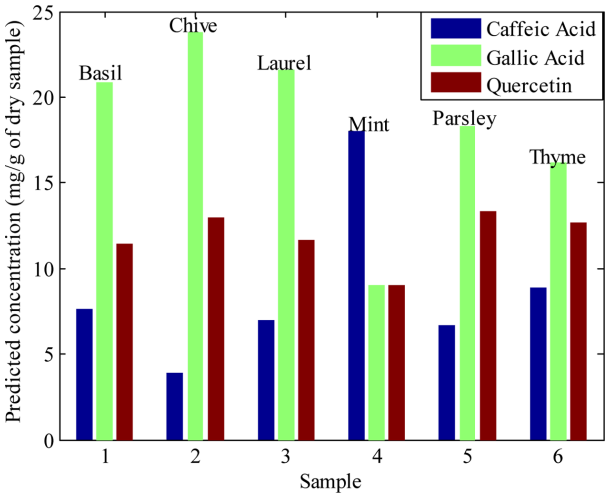

***Figure* 6.** Predicted concentration of CA, GA and Q in the six aqueous herb extracts.

Application of the PLS Model to the Prediction of the CA, GA and Q Quantities Contained in Natural Samples

In order to verify the robustness of the PLS model used; we applied it on the spectral data of six aqueous herb extracts. The UV spectra obtained from the different solutions were shown in *Fig*. 5.

The UV spectra of mint, thyme, basil, and laurel have two absorption maxima (at 284 nm and 324 nm), and the shape of these spectra look like that of caffeic acid. The spectrum of parsley has two absorption maxima one at 266 nm like gallic acid, and another at 332 nm which resembles that of quercetin. The chive spectrum shows two absorbance maxima; one at 266 nm like gallic acid, and the other 284 nm like caffeic acid. The band intensities of the chive spectrum are the smallest, whereas those of the mint spectrum are the greatest. The predicted concentrations of CA, GA, and Q in the studied herbs, using the PLS model, were presented in *Fig*. 6, they were found to be 3.918–18.055, 9.014–23.825, and 9.040–13.350 mg/g of dry sample, respectively. The mean content (mg/g of dry sample) in descending order was GA (18.307), Q (11.85), and CA (7.214).

The highest levels of CA, GA, and Q were found respectively in mint, chive, and parsley. The lowest levels of GA and Q were found in mint, and that of CA was found in chive.

Caffeic acid contents announced in other works were found to be 1.2–60 mg/100 g of dry sample in Greek aromatic plants,[34] 0–0.4 mg/g in thyme and spearmint,[35] 5.8–30 mg/100 g in thyme, and 2.8 mg/100 g in sweet basil. Gallic acid contents in other works were found to be 11.7, 0.7, and 0.9 mg/100 g fresh weight in thyme, parsley, and spearmint, respectively. Quercetin contents in other works were found to be 9 mg/kg in chives,[36] generally below 10 mg/kg in most vegetables,[37] 0–170 mg/100 g in Danish fresh herbs,[38] 1.3 mg/100 g and 10.4 mg/100 g in parsley and chives, respectively.[39] Some of the amounts of caffeic acid, gallic acid, and quercetin found in this study were different compared with other studies. This gap could be explained by the difference in the varieties and growing conditions of the herbs analyzed in these studies.

CONCLUSION

The “Caffeic acid - Gallic acid - Quercetin” mixture is a diffcult system to analyse due to the high spectral overlapping observed between the UV absorption spectra of these phenolic compounds. The determination of these polyphenols using UV visible spectrometry and PLS

regression was established with a good prediction ability for synthetic mixtures. The results obtained establish that PLS modelling can be used successfully for the simultaneous determination of caffeic acid, gallic acid and quercetin in their mixture. This work also shows that CA, GA and Q contents, obtained using the PLS model on UV spectral data of infusion extracts of the studied herbs were higher compared to the results obtained by other authors who used different experimental methods. This may be related to the high amount of flavonoids and phenolic acids in the herbs used in this work. Another possible explanation is the difference of the provenance of the herbs and the analytical methods that were used in this study compared to the ones used in the other research works.

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