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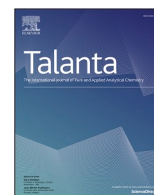

Application of HPLC-UV combined with chemometrics for the detection and quantification of 'true cinnamon' adulteration

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A B S T R A C T

Cinnamon is one of the most popular spices used in cuisines worldwide. Among its different species, Ceylon cinnamon ("true cinnamon") is the one with the most health benefits due to its high concentration in the antioxidant eugenol and the ultra-low content of the hepatotoxic compound coumarin. However, the higher price of Ceylon cinnamon makes it vulnerable to fraudulent adulteration with more economic species of cinnamon, such as Cassia and Saigon. Thus, for the detection of frauds in cinnamon samples, a HPLC-UV method was developed for the determination of 4 characteristic cinnamon compounds: eugenol, cinnamaldehyde, coumarin and cinnamic acid. The obtained data were analyzed by PLS to attain not only the authentication of cinnamon species but also the detection and quantification of partial adulterations. Several mixtures prepared in the laboratory using different cinnamon powder samples considered 'pure' Ceylon, Cassia or Saigon were tested, concluding that the proposed approach allows a clear identification of Ceylon cinnamon and a suitable quantification of the Ceylon: non-Ceylon ratio regardless of the commercial sample selected (RMSE <0.06 for both training and test sets).

1. Introduction

Cinnamon is one of the oldest known and most popular spices due to its uses as aromatic condiment, flavoring additive and natural preservative, as well as to its potential health-promoting properties (e.g. antioxidant, anti-inflammatory, antidiabetic, anticancer, antimicrobial, and antilipemic) [1,2]. Cinnamon is obtained from the internal bark of different types of trees of the genus *Cinnamomum*, which translates into a wide variety of cinnamon species. One of the most known species is Ceylon cinnamon, which is usually referred to as "true cinnamon" and is obtained from the bark of the *Cinnamomum verum,* originally from Sri Lanka and Madagascar. Other main species of cinnamon are *Cinnamomum cassia* (also called Chinese cinnamon) from China, and *Cinnamomum loureiroi* (also known as Vietnamese or Saigon cinnamon) from Vietnam. Each species of cinnamon has a chemical composition and characteristic properties depending on its geographical origin, climate and cultivation methods. Thus, the commercial cost of the different species of cinnamon is associated with their quality, being Ceylon cinnamon the most expensive one due to its chemical composition, high quality, demonstrated health benefits, and ultra-low levels of toxic compounds [3,4].

The growing demand and popularity of cinnamon has led to an increase in fraudulent adulteration, allowing higher profits to be made at the expense of the quality of the product. It should be noted that this practice is more widespread in ground cinnamon because its powdered format hinders the visual detection of adulteration, whereas consumers can more easily distinguish the difference in cinnamon sticks. For example, rolled bark of Ceylon cinnamon is thinner, multilayered and easier to break and grind as compared to the thicker and more robust bark of Cassia cinnamon. Thus, Ceylon cinnamon (especially in ground form) is prone to adulteration with the lower priced Cassia cinnamon, being often mislabeled and sold as 'true cinnamon'. Other common

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adulterants of ground cinnamon can be flour and other bulking agents such as coffee husks, since they have the same color and texture in their powdered format [2,5]. This fraudulent adulteration is a serious public health problem, as it may result in high concentrations or prolonged exposure to certain compounds that can have negative effects on human health (e.g. carcinogenic) or even be fatal. Moreover, product mislabeling can easily lead to severe allergic reactions [6]. Thus, a good quality control is required to ensure the traceability of the product and to have product guarantees for both consumers and stakeholders of the supply chain.

Among the most common analytical techniques to authenticate and characterize spices one can find gas chromatography coupled to a flame ionization detector (FID) or single/tandem mass spectrometry (MS (/MS)), and liquid chromatography coupled to MS(/MS), ultraviolet–visible detector (UV–vis), fluorimetric detector (FD) or electrochemical detector (ED) [7]. In the particular case of cinnamon, apart from the most common chromatographic techniques, spectroscopic methods such as Fourier-transform infrared (FT-IR) spectroscopy, Fourier transform near-infrared (FT-NIR) and nuclear magnetic resonance (NMR) [8–10], and direct analysis in real time (DART) coupled with QToF-MS [11] have also proved to be effective as screening methods to authenticate cinnamon species.

In food authentication problematics in which one specific adulterant is suspected (e.g. Ceylon cinnamon being adulterated with Cassia cinnamon), target analysis provides high selectivity and sensitivity. Furthermore, target analysis allows for a quantitative approach, in which an adulteration is not only detected but also quantified [12]. For this purpose, characteristic biomarkers are selected and quantified. In the case of cinnamon, cinnamaldehyde and its metabolic product cinnamic acid are interesting choices because cinnamaldehyde accounts for up to 90 % of the composition of the essential oil responsible for cinnamon taste [13]. In addition, previous studies have also reported eugenol or coumarin as other potential biomarkers [10,13]. In this sense, it should be noted that an important difference between Ceylon cinnamon and Cassia cinnamon is the content of coumarin, a naturally occurring substance found in different plant sources. Ceylon cinnamon has an average concentration of 2–5 mg kg^{-1} of coumarin, whereas the coumarin content in Cassia cinnamon is approximately 2000–5000 mg kg^{-1} [8]. This is a particularly relevant difference because coumarin was declared as hepatotoxic and carcinogenic by the European Food Safety Authority (EFSA), who established a daily intake limit (TDI) of 0.1 mg kg^{-1} of body weight [14]. On the other hand, it should also be pointed out that eugenol is a major compound in Ceylon cinnamon but not in Cassia cinnamon, making it a characteristic biomarker to distinguish the different species of cinnamon [15,16].

In view of the foregoing, this work evaluates the suitability of employing only these four compounds (i.e. cinnamaldehyde, cinnamic acid, coumarin and eugenol) for the authentication of cinnamon species, detection of frauds and the quantification of the adulteration in commercial cinnamon powder samples. For this purpose, an analytical method based on liquid chromatography with UV–vis detection after a very fast and simple sample treatment (extraction with methanol) was developed and analytically validated for the determination of these four characteristic compounds. The obtained data were treated by partial least squares (PLS) to achieve not only the discrimination among cinnamon species but also the detection and quantification of partial adulterations. Special emphasis was placed in Ceylon cinnamon adulteration with Cassia cinnamon, although adulteration with Saigon cinnamon was also considered to evaluate the influence of other cinnamon species. This method was successfully tested with several mixtures prepared in the laboratory using different commercial samples considered 'pure' Ceylon, Cassia or Saigon.

2. Experimental section

2.1. Chemicals and solutions

For the preparation of mobile phase, formic acid (98 %, PanReac AppliChem, Barcelona, Spain), methanol (HPLC Gradient rate, ≥99.9 %, Fisher Scientific, Geel, Belgium) and Milli-Q water (reference A+, Millipore, France) were used. The standards of coumarin and eugenol were provided by Sigma Aldrich (Steinheim, Germany), whereas cinnamaldehyde and cinnamic acid were purchased from Thermo Fisher (Kandel, Germany). For each compound, a concentrated standard stock solution was prepared weekly in methanol and stored at 4 °C. Intermediate solutions were prepared daily by dilution with methanol.

2.2. HPLC-UV analysis

HPLC-UV analysis was performed using a chromatographic system comprised of an Agilent 1200 Series instrument (Palo Alto, CA, USA) that included a quaternary pump (G1311A), a vacuum degasser (G1322A), an autosampler (G1329A), an ultraviolet–visible detector (G1314B) and the Agilent ChemStation software in a personal computer to acquire and process the data. The separation was performed in a Kinetex® column (5 μm C18 100 Å, 100 × 4.6 mm) provided by Phenomenex (Torrance, CA, USA). Chromatograms were acquired at 280 nm using an injected volume of 10 μL and a flow rate of 1 mL min^{-1}. The elution gradient protocol combined 0.1 % formic acid in Milli-Q water

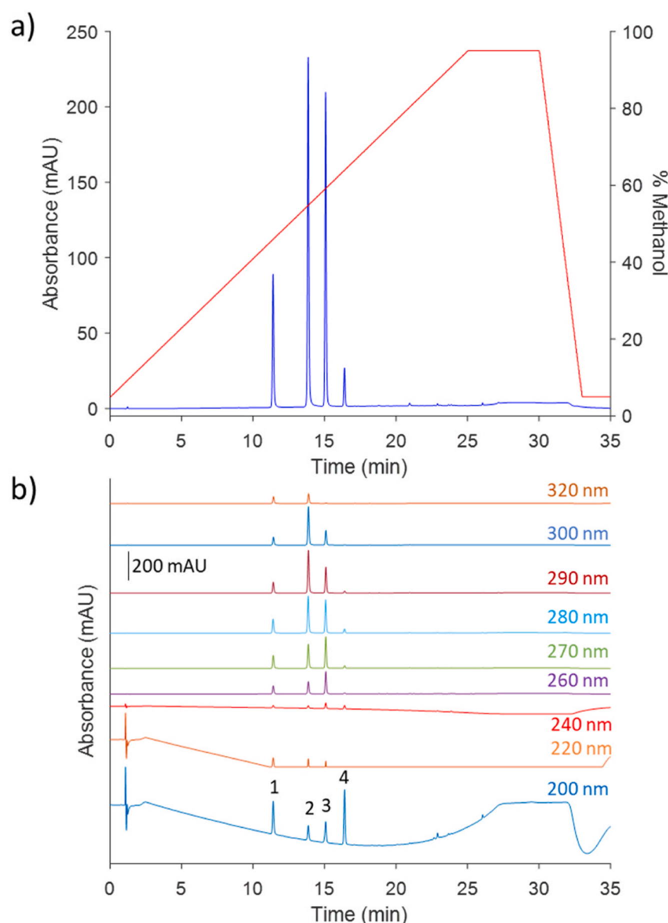

Fig. 1. a) Chromatographic profile (blue line, left axis) obtained with the optimized gradient elution (red line, right axis) for the separation of coumarin (1), cinnamaldehyde (2), cinnamic acid (3), and eugenol (4) at 15 mg L^{-1} and recorded at 280 nm. **b)** Optimization of the wavelength for UV-detection from 200 to 320 nm under the same conditions as in (a).

Table 1
Quality parameters for the optimized HPLC-UV method (Level 1 = 100 mg L^{-1}; Level 2 = 800 µg L^{-1}).

Compound		Coumarin	Cinnamaldehyde	Cinnamic acid	Eugenol
Sensitivity (mAU s mg^{-1} L)		39.5 (0.1)	80.7 (0.2)	70.5 (0.2)	9.92 (0.03)
R^2		0.9997	0.9997	0.9996	0.9994
Linear Range (mg L^{-1})[a]		0.08–550	0.1–250	0.08–250	0.02–800
ILOD (µg L^{-1})		25.2	32.7	25.1	5.0
Repeatability (RSD,%)	**Level 1**	1.0	1.1	1.0	1.0
	Level 2	0.4	0.4	0.6	1.4
Reproducibility (RSD,%)	**Level 1**	3.0	2.5	2.8	3.0
	Level 2	1.4	4.2	0.6	1.6
Trueness (% Error)	**Level 1**	0.7	0.8	0.5	0.6
	Level 2	0.2	0.3	0.3	0.3

[a] Linear range with the ILOQ as the lower value.

(solvent A) and methanol (solvent B) as follows: 0–25 min, linear gradient from 5 to 95 % solvent B; 25–30 min, isocratic elution at 95 % solvent B; 30–33 min, from 95 to 5 % solvent B; and 33–35 min at 5 % solvent B for re-equilibration of the column between injections.

2.3. Samples and sample preparation

A total of 95 samples of different species of cinnamon were analyzed (Table S1). In particular, samples were distributed among the different cinnamon species as follows: 25 samples of Ceylon cinnamon (16 powder and 9 sticks), 25 samples of Cassia cinnamon (20 powder and 5 sticks), 20 samples of Saigon (15 powder and 5 sticks) and 25 samples of undefined cinnamon (13 powder and 12 sticks). All samples were commercially available and collected from local supermarkets and online retailers. Cinnamon sticks were reduced to a fine powder by means of a coffee grinder.

For chromatographic analysis, 0.5 g of powdered sample were dissolved in 2 mL of methanol and vortexed for 1 min. The samples were then sonicated for 15 min and centrifuged for 45 min at 4500 rpm. The supernatant obtained was extracted and filtered through 0.22 µm nylon filters and methanol was added up to 2 g and stored at −18 °C until analysis.

The obtained samples were injected directly and after further dilution with methanol according to the dilution factors displayed in Table S1 to ensure that all analytes fell into the corresponding linear range. All samples were analyzed randomly and in triplicate. Before the injection of any sample set, 6 blanks of methanol were analyzed to stabilize the HPLC and two more blanks were also analyzed every fifteen samples for control purposes.

For the quantification of partial adulteration, cinnamon samples were adulterated in the laboratory by mixing different ratios of the 3 pure species of cinnamon, according to the experimental design shown in Table S2, which comprises a total of 49 mixed samples. Samples belonging to the training set (M1 to M24) and test set 1 (N1 to N16) were prepared by mixing samples C34, C48 and C45, which were considered pure Ceylon, pure Cassia and pure Saigon, respectively. On the other hand, samples belonging to test set 2 (P1 to P9) were prepared from samples C33 (Ceylon), C50 (Cassia), and C42 (Saigon). All samples were analyzed randomly and in triplicate.

2.4. Data treatment

Chemometric analysis was performed in PLS_Toolbox from Eigenvector Research [17]. Principal component analysis (PCA) was employed for exploratory purposes whereas partial least squares (PLS) models were built for the quantification of adulterations. In both methods, the content of the four considered compounds (coumarin, eugenol, cinnamaldehyde and cinnamic acid), expressed as mg g^{-1}, was used as input. Data were first autoscaled to account for differences of magnitude and variance. 2 principal components (PC) were employed in the PCA model.

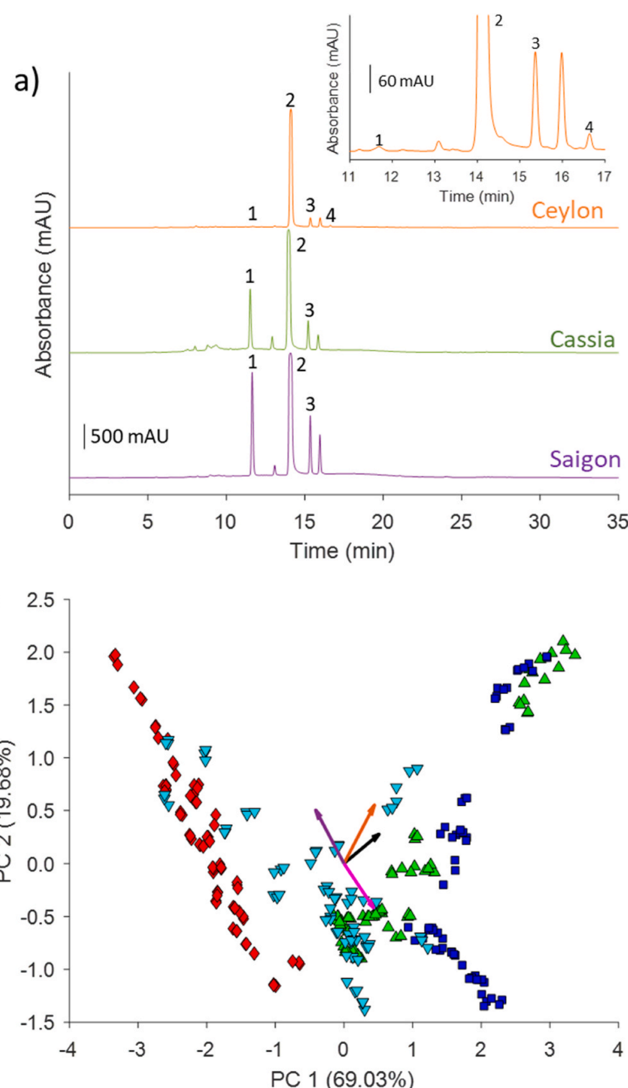

Fig. 2. a) Representative chromatograms of each type of cinnamon recorded at 280 nm for the separation of coumarin (1), cinnamaldehyde (2), cinnamic acid (3), and eugenol (4). **b)** Biplot of the scores and loading diagrams obtained in the PCA analysis of cinnamon samples. Symbols indicate the scores obtained for each commercial sample (Ceylon, ♦; Cassia, ■; Saigon, ▲; and not specified cinnamon, ▽) whereas arrows represent the loadings for each characteristic compounds studied (coumarin in black, cinnamaldehyde in orange, cinnamic acid in magenta, and eugenol in purple).

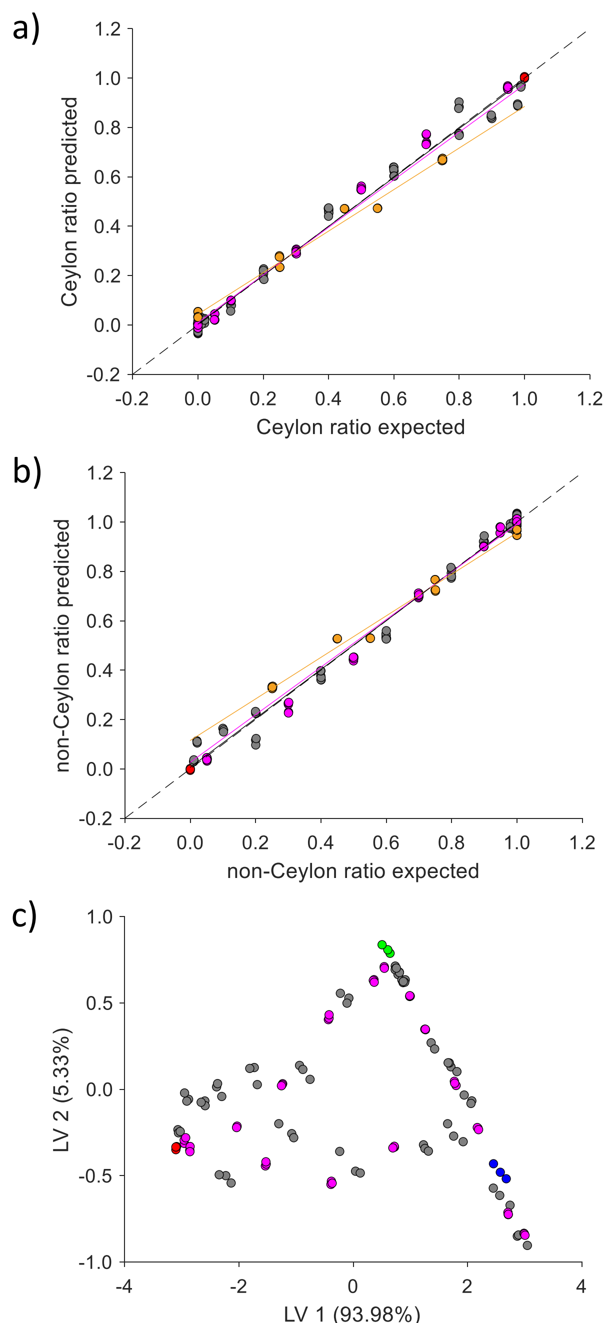

Fig. 3. Performance of PLS model for the authentication of cinnamon: comparison graphs of predicted *vs.* expected ratio for Ceylon **(a)** and non-Ceylon **(b)**; and scores diagram **(c)**. Legend: training set (●), test set 1 (●), test set 2 (●) set, pure Ceylon (●), pure Cassia (●), and pure Saigon (●). Dashed lines in (a) and (b) represent theoretical ideal lines (y = x).

Numerical quantification of the degree of adulteration was assessed at two levels: (i) adulteration of Ceylon samples with either Saigon or Cassia samples (*i.e.* two classes corresponding to Ceylon and non-Ceylon); and (ii) adulteration among the three species of cinnamon (*i. e.* three classes corresponding to Ceylon, Cassia and Saigon). The number of latent variables (LV), selected according to the first minimum in the average classification error obtained in cross validation (venetian blinds, 10 splits and 1 sample per blind), were 2 and 3, respectively.

Each PLS model was validated using two different test sets (Table S2). The first set (test set 1) included samples prepared by mixing the same commercial cinnamon samples employed in the preparation of the training set (C34, C48 and C45) and considered a 60:40 ratio for samples employed in the training and test sets. Training set 2 was composed of samples obtained from the combination of different commercial samples (C33, C50 and C42) and accounted for a 75:25 training: test ratio.

3. Results and discussion

3.1. HPLC-UV optimization

First of all, in order to establish the chromatographic method, it was necessary to optimize the chromatographic separation based on four characteristic and important constituents of cinnamon: coumarin, cinnamaldehyde, cinnamic acid and eugenol [10,13]. The objective was to attain an optimal separation of the compounds in the shortest possible analysis time and to achieve the highest efficiency and sensitivity of the chromatographic peaks to avoid both confusions between compounds and co-elutions. As the considered compounds have a very similar structure, it was necessary to use an elution gradient for the separation.

The optimal separation was reached by applying the elution gradient protocol detailed in section 2.2. The chromatogram obtained at the optimized gradient conditions for a 15 mg L^{-1} standard mixture of the considered compounds is shown in (Fig. 1a). As it can be observed, a good separation was obtained with a total time of 35 min.

The optimization of the UV detection step is also very important for the development of the chromatographic method. Thus, the effect of the wavelength over the peak area of the studied compounds was evaluated in the range from 200 to 320 nm (Fig. 1b). As can be seen in Fig. 1b, at 280 nm all the peaks related to the four considered compounds can be easily detected with a very good baseline, and most of the achieved peaks are higher than those obtained at the other tested wavelengths. Consequently, a wavelength value of 280 nm was chosen as optimal for further experiments.

3.2. Validation of HPLC-UV method

Once the HPLC-UV method is optimized, the validation parameters for the four considered compounds were calculated (Table 1). Linear calibration curves were carried out in triplicate at the above-established conditions by determining increasing concentrations of coumarin, cinnamaldehyde, cinnamic acid and eugenol varying from 0.05 mg L^{-1} to 800 mg L^{-1}. Very good linearities with correlation coefficients (R^2) higher than 0.9994 were achieved for the four studied compounds, which were maintained up to 550 mg L^{-1} for coumarin, 250 mg L^{-1} for cinnamaldehyde and cinnamic acid, and 800 mg L^{-1} for eugenol. Sensitivities, determined from the slope of the calibration lines, varied from 9.92 to 80.7 mAU s mg^{-1} L depending on the considered compound. Instrumental detection limits (ILODs), based on a signal-to-noise ratio of 3:1, were computed using standard solutions prepared in methanol at low concentration levels, and values between 5.0 μg L^{-1} and 32.7 μg L^{-1} were achieved depending on the considered compound. Instrumental quantification limits (ILOQs), which were based on a signal-to-noise ratio of 10:1, were in the range of 16.7–109.0 μg L^{-1} depending on the target analyte.

The performance of the HPLC-UV method was also evaluated in terms of repeatability (intra-day) and reproducibility (inter-day) at two different concentration levels (100 mg L^{-1} and 800 μg L^{-1}) as seen in Table 1. Repeatability was computed for ten repetitive measurements whereas reproducibility was calculated on three different days within a series of ten repetitive measurements. The precision parameters showed relative standard deviation (RSD%) values between 0.4 and 1.4 % for repeatability and from 0.6 to 4.2 % for reproducibility, depending on both the measured level and the considered compound.

The trueness of the method, expressed as relative error (%), was assessed by comparing spiked target concentrations with those obtained using external calibration at the two concentration levels (100 mg L^{-1}

Table 2
Main parameters of the regression lines obtained in the comparison of predicted and expected values of the training and test subsets of cinnamon in PLS models built considering either two (Ceylon, non-Ceylon) or three (Ceylon, Cassia, Saigon) classes.

	Model with 2 classes		Model with 3 classes		
	Ceylon	Non-Ceylon	Ceylon	Cassia	Saigon
Training set					
Slope	0.99 (0.01)	0.990 (0.006)	0.99 (0.01)	0.91 (0.03)	0.94 (0.03)
Intercept	0.003 (0.006)	0.006 (0.009)	0.003 (0.006)	0.03 (0.02)	0.02 (0.01)
R^2	0.990	0.990	0.990	0.914	0.941
RMSE	0.037	0.037	0.037	0.112	0.092
Test set 1					
Slope	1.05 (0.01)	1.05 (0.01)	1.05 (0.01)	0.99 (0.03)	1.04 (0.03)
Intercept	−0.004 (0.004)	−0.044 (0.007)	−0.005 (0.004)	−0.09 (0.02)	0.07 (0.01)
R^2	0.996	0.996	0.996	0.952	0.961
RMSE	0.030	0.030	0.030	0.122	0.109
Test set 2					
Slope	0.84 (0.02)	0.84 (0.02)	0.84 (0.02)	0.9 (0.2)	1.68 (0.09)
Intercept	0.044 (0.007)	0.12 (0.01)	0.041 (0.007)	−0.22 (0.08)	0.05 (0.04)
R^2	0.991	0.991	0.991	0.465	0.931
RMSE	0.052	0.052	0.052	0.377	0.363

and 800 μg L^{-1}), considering 5 replicates. The overall relative errors ranged from 0.2 to 0.8 % depending on both the spiked level and the considered compound.

With these results, it can be concluded that the proposed method is suitable for cinnamon analysis as it has good precision and trueness, excellent linearity and high sensitivity, as well as low ILOQ and ILOD values.

3.3. Exploratory analysis

Once the HPLC-UV method was optimized and validated, the applicability of this method for the determination of coumarin, cinnamaldehyde, cinnamic acid and eugenol in samples of different species of cinnamon was evaluated. For this purpose, the sample treatment protocol was optimized considering three replicates of three pure samples of cinnamon (Ceylon (C34), Cassia (C48) and Saigon (C45)) and aiming for a reproducible yet simple extraction method. As a first approximation, 2 mL of methanol were added onto 0.5 g (accurately weighed) of cinnamon sample and subjected to stirring with a vortex mixer for 1 min and further sonication for 15 min. This protocol demonstrated an acceptable reproducibility for the extraction of cinnamaldehyde (between 1.7 and 4.9 % depending on the considered sample) but failed to provide a reproducible extraction for the other three compounds (RSD (%) values around 8–10 %). Thus, an extra extraction step was added consisting on 45 min of centrifugation at 4500 rpm. This last step significantly improved the reproducibility, obtaining RSD (%) values below 4 % for all analytes, which is acceptable for target analysis purposes.

The modified extraction protocol was implemented in the analysis of 95 samples of cinnamon pertaining to the three different species (Table S1). All samples were analyzed in triplicate at the optimal chromatographic conditions, giving rise to a total of 285 result values. Fig. 2a displays representative chromatograms acquired for cinnamon sample extracts corresponding to Ceylon, Cassia and Saigon species. The first thing to be noted is that the composition of the different species of cinnamon is not the same, neither with regard to the detected components, nor in terms of their concentration. As it is shown in Fig. 2a and Table S3, only Ceylon extracts contained the four considered compounds, as the peak corresponding to eugenol was not usually observed in the chromatograms of Cassia and Saigon samples. On the other hand, the presence of coumarin in Ceylon samples is often minimal, whereas the content of coumarin in Cassia and Saigon samples is very significant and about two orders of magnitude higher as compared to Ceylon samples. Regarding the other studied compounds, the content of both cinnamaldehyde and cinnamic acid is frequently higher in Saigon and Cassia extracts than in Ceylon samples.

Therefore, the observed differences in the chromatograms can be used by pattern recognition techniques such as PCA to discriminate among different species of cinnamon. A PCA study was performed with a data set integrated by the concentration of the four target compounds obtained from the 285 extracted samples. Fig. 2b shows the combined scores and loadings plot obtained, with principal components 1 and 2 accounting for 69.03 and 19.68 % of the variance explained, respectively. Regarding the scores, it can be observed that Ceylon samples are easily discriminated from Cassia and Saigon samples, as they present significantly lower values of PC1. However, the clusterization of Cassia and Saigon samples is not as clear, as there is some overlap among the two species. Among these two groups, it appears that the format of the sample (stick or powder) has a stronger effect on the scores, since the group located at the top right corner of the plot is only composed of stick cinnamon, either Cassia or Saigon. On the other hand, commercial samples that did not specify the cinnamon specie in the product label are distributed along the entire plot since they can come from different cinnamon species, but with a higher prevalence near the Cassia/Saigon region. This was actually expected as Ceylon cinnamon is the most expensive specie and, therefore, producers will strongly promote its appropriate labeling. In terms of loadings, only eugenol presents a negative value of PC1, indicating that eugenol concentration is higher in Ceylon cinnamon whereas coumarin, cinnamaldehyde and cinnamic acid are found in higher concentrations in Cassia and Saigon cinnamon. Indeed, this agrees with the results reported in Table S3.

3.4. Authentication of cinnamon samples

The results obtained in the exploratory analysis demonstrated that eugenol, coumarin, cinnamaldehyde and cinnamic acid present distinguishable enough contents in Ceylon cinnamon and non-Ceylon cinnamon to be able to differentiate among them. Nevertheless, adulteration cases usually consist in partial adulteration and, therefore, an ideal authentication method for cinnamon should also be able to quantify the adulteration degree. For this purpose, a PLS model was built based on adulterated samples prepared in the laboratory consisting of binary mixtures as explained in section 2.3 and Table S2. It should be noted that for this model only powdered cinnamon was considered as it is more vulnerable to adulteration than stick cinnamon and that samples C34, C48 and C45 were employed as pure Ceylon, Cassia and Saigon cinnamons. A total of 24 samples (measured in triplicate) was employed in the training of the model and the optimal number of latent variables (LVs) was 2, with LV1 and LV2 accounting for a 93.98 and 5.33 % of the variance of X explained, respectively. The prediction capability of the model was assessed by an external validation, which employed further binary mixtures of samples C34, C48 and C45 (see Table S2, test set 1). The results obtained are displayed in Fig. 3a and b, which compare the

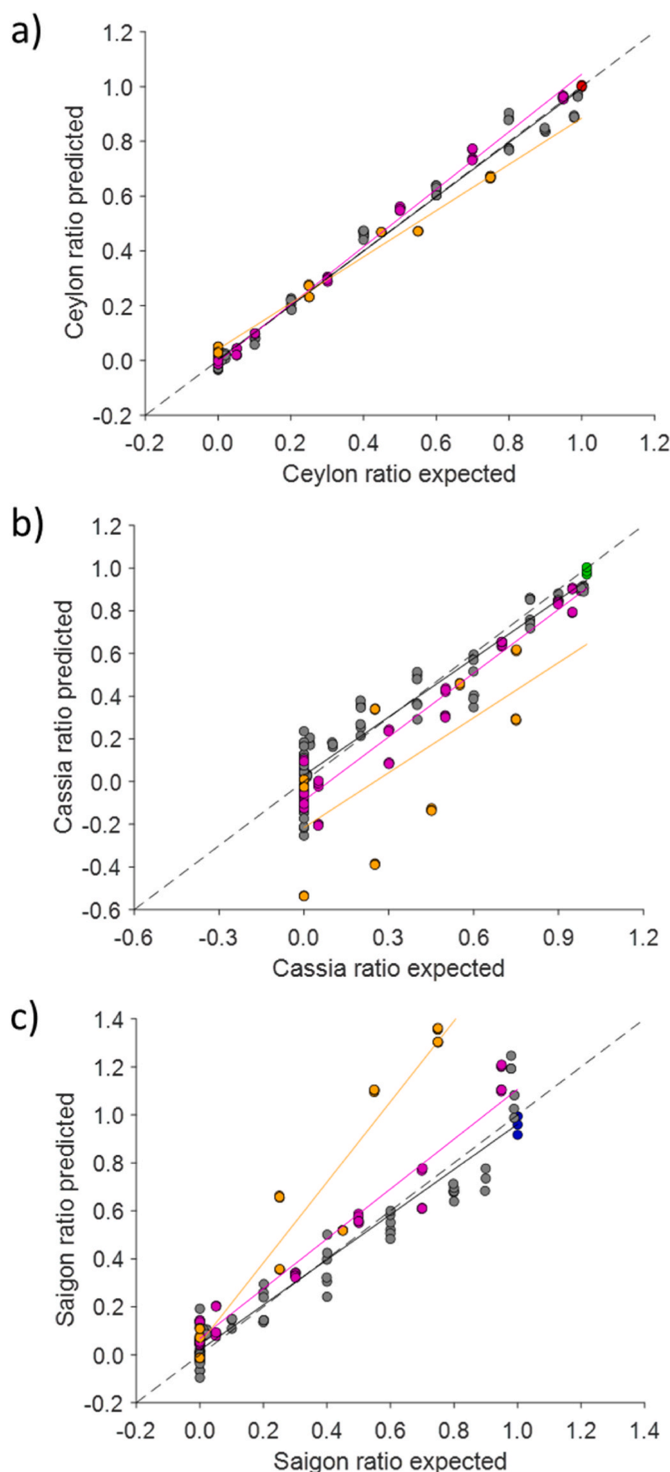

Fig. 4. Comparison graphs of predicted *vs.* expected ratio for Ceylon **(a),** Cassia **(b)** and Saigon **(c)**. Legend: training set (●), test set 1 (●), test set 2 (●) set, pure Ceylon (●), pure Cassia (●), and pure Saigon (●). Dashed lines represent theoretical ideal lines (y = x).

predicted and expected ratios. Additionally, regression parameters are provided in Table 2. As it can be observed, the fitting of both training set (black) and test set 1 (magenta) were satisfactory, with regression lines almost identical to the ideal line (y = x), regression coefficients close to 1 and low root mean square errors (RMSE). Nevertheless, in order to ensure that this model can be extrapolated to any commercial sample and is not dependent on the three samples employed in the preparation of the binary mixtures, a second external validation was carried out with a new test set 2 prepared from a different set of Ceylon, Cassia and Saigon cinnamons (samples C33, C50 and C42). As observed in Table 2 and Fig. 3, the regression obtained for test set 2 is close to that of set 1, confirming that the developed model is able to predict the ratio Ceylon and non-Ceylon cinnamons regardless of the commercial sample selected.

A closer inspection of the scores plot (Fig. 3c) revealed a sample distribution resembling a triangle with the pure samples at each vertex. This distribution is typical for PLS models with binary mixtures of three components and, therefore, the possibility to be able to quantify not only the Ceylon ratio but also the ratio for Cassia and Saigon species was evaluated. For this purpose, a new PLS model was built employing the same training and test sets but considering three dependent variables (Ceylon ratio, Cassia ratio and Saigon ratio). In this case, the optimal number of LVs was 3, with LV1, LV2 and LV3 accounting for 94.02, 5.29 and 0.40 % of the variance of X explained, respectively. Likewise, this model was able to correctly predict the Ceylon ratio (Fig. 4a), with regression values almost identical to those obtained in the previous model (Table 2). Regarding Cassia (Fig. 4b) and Saigon ratios (Fig. 4c), the regression parameters were not as ideal as in the case of Ceylon but still appropriate for quantification purposes, with and intercept close to zero, a slope and regression coefficient close to 1, and low RMSE values (Table 2). However, validation with test set 2 revealed an insufficient prediction capability for Saigon and Cassia ratios, which indicates that quantification of Ceylon ration is independent of the commercial sample but Saigon and Cassia rations are highly dependent of the samples employed in the training. Altogether, the results obtained indicate that the quantification of eugenol, coumarin, cinnamaldehyde and cinnamic acid allows the quantification of adulteration degrees in Ceylon cinnamons but is not enough to distinguish among other cinnamon species such as Cassia and Saigon, which present a much similar composition [3]. Nevertheless, it should be considered that adulteration of Ceylon cinnamon is the most probable scenario for cinnamon adulteration since Ceylon is the healthiest and most expensive specie.

4. Conclusions

The combination of liquid chromatography with chemometric techniques has proved to be a valuable strategy for the authentication of cinnamon species, detection of frauds and the quantification of the adulteration in commercial cinnamon powder samples. Although there are some studies related to the authentication of cinnamon samples, this study is, as far as we know, the first that has explored the use of HPLC-UV combined with chemometrics not only to discriminate among cinnamon species but also to detect and quantify partial adulterations.

Cinnamon samples were subjected to a fast and simple sample extraction procedure by sonication and centrifugation, which demonstrated a very good reproducibility for the extraction of eugenol, coumarin, cinnamaldehyde and cinnamic acid. Its determination was carried out in reversed-phase chromatography using a C18 column and a universal gradient elution profile (from 5 to 95 % methanol in 25 min, followed by 5 min at 95 % methanol, and then 5 extra minutes to back to initial conditions for column re-equilibration). The developed HPLC-UV method was analytically validated for the determination of these four target compounds, showing excellent linearities up to 550 mg L^{-1} for coumarin, 250 mg L^{-1} for cinnamaldehyde and cinnamic acid, and 800 mg L^{-1} for eugenol. Moreover, high sensitivities and ILODs between 5.0 μg L^{-1} and 32.7 μg L^{-1} were attained depending on the target compound. Repeatability and reproducibility, calculated as percentage of RSD, were lower than 1.4 % and 4.2 %, respectively for all considered compounds and levels, whereas the trueness was always in the range of 0.2–0.8 %.

From the PCA study it can be concluded that Ceylon samples are easily discriminated from Cassia and Saigon samples, whereas the clusterization of Cassia and Saigon samples is not as well-defined since

they present a much similar composition. From the loadings it can be stated that coumarin, cinnamaldehyde and cinnamic acid concentrations are higher in Cassia and Saigon cinnamon whereas eugenol is found in higher concentrations in Ceylon cinnamon.

Regarding the detection and quantification of partial adulterations, it can be concluded that the content of eugenol, coumarin, cinnamaldehyde and cinnamic acid allows a clear identification of Ceylon cinnamon and a suitable quantification of the Ceylon: non-Ceylon ratio regardless of the commercial sample employed in the preparation of test mixtures (RMSE lower than 0.06 in all cases). Nevertheless, the difference between Cassia and Saigon cinnamon is much more subtle and, although preliminary results were promising in their separation, final results indicated that the quantification of these two species is highly dependent on the considered commercial samples and is, therefore, not reliable enough. Thus, the proposed method is suitable for the most common case of adulteration in cinnamon (i.e. adulteration of Ceylon cinnamon with either Cassia or Saigon) but more compounds should be added in order to clearly distinguish between Cassia and Saigon cinnamons.

CRediT authorship contribution statement

Josep Pages-Rebull: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Gemma Sagristà:** Formal analysis. **Clara Pérez-Ràfols:** Writing – original draft, Visualization, Supervision, Methodology, Formal analysis, Conceptualization. **Núria Serrano:** Writing – original draft, Visualization, Supervision, Methodology, Formal analysis, Conceptualization. **José Manuel Díaz-Cruz:** Writing – review & editing, Visualization, Validation, Resources, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2024.125676.

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