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LC-DAD Combined with Spectral Deconvolution (LC-DAD/SD) for the Analysis of Some Diterpene Esters in Arabica Coffee Brews

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Abstract

In this manuscript, the separation of four kahweol esters and four cafestol esters that are present in Arabica coffee brews was investigated using HPLC/DAD. Those compounds could not be baseline separated in a single chromatogram using RP-LC but the kahweol esters could be selectively detected by setting the wavelength at 290 nm. In this case the four kahweol esters were baseline separated allowing for their quantification. Such approach was not possible for the cafestol esters and spectral deconvolution was used to obtain deconvolved chromatograms that were specific to cafestol and kahweol esters respectively. In each of those chromatograms, the 4 esters were baseline separated allowing for the quantification of the eight targeted compounds. Because kahweol esters could be quantified either using the chromatogram obtained by setting the wavelength at 290 nm or using the deconvolved chromatogram, those compounds were used to compare the analytical performances. Slightly better *LOD* were obtained using the deconvolved chromatogram (average *LOD* of 5.7 mg/L against 6.7 mg/L). Identical concentrations were found in a real sample with both approaches. The peak areas of the different diterpene esters in the deconvolved chromatograms were proven to be repeatable with an average intra-day repeatability of 0.8 % (6 replicates) and an inter-day repeatability of 1.0% (3 successive days, two replicates each day). This work demonstrates the accuracy of spectral deconvolution in conjunction with HPLC-DAD (HPLC-DAD/SD) to mathematically separate co-eluting compounds using the full spectra recorded by the DAD.

Keywords: Cafestol esters, Kahweol esters, Diode array, Matlab, Spectral deconvolution, Sum squared residuals

1. Introduction

In separation science, the term hyphenated technique describes the combination of a separation technique (LC, GC, CE...) and a spectrometric technique (DAD, MS, FTIR...) that is used as the detector [1]. The recorded chromatographic data are often refer as first order data [2] and they are (or can be arranged into) a two dimensional table with the time axis in one dimension, the spectrum axis in the other dimension and the amplitude of the signal as responses. The simplest way to work with such a dataset is by generating mono-dimensional traces (chromatogram, electropherogram, MS spectra, UV spectra…) that are obtained by taking the responses as a function of times at a particular spectral coordinate (chromatogram) or taking the responses as a function of the spectral coordinates at a given time (spectra). Not only this allows obtaining spectra at different times for identification purposes but also allows obtaining chromatograms in which the peak areas can be modulated by selecting the spectral coordinate [3]. Hyphenated MS techniques are probably the best combination for such applications [4-9] and it is often possible with those instruments to choose a spectral coordinate at which one or more compounds of interest will be present in the chromatogram but where potentially interfering species will be transparent. However, MS instruments remain costly to buy and run. Diode array detectors (DAD), due to their low prices and good precision are probably the most common hyphenated detector but their low spectral selectivity does not often allow resolving co-elution problems as easily as with a MS detector. The use of chemometric approaches in conjunction with the DAD dataset can allow the mathematic separation of co-eluted species [2,10-15], where mathematical separation refers to the deconvolution of the dataset in which co-elution (or co-migration) occurs to a series of simpler chromatograms by mathematical means.

Spectral deconvolution (SD) is a technique that can be applied to any spectrometric dataset [16]. The key assumption in this approach is that at any time, the spectrum that is recorded by the detector is a linear

combination of the spectra of every compound that are present in solution. This assumption is true as long as the detector is used within its linear range and can be expressed mathematically as [14,16,17]

$$\mathbf{Y}(t) = \mathbf{X}\beta(t) + \varepsilon(t) \quad (1)$$

where $\mathbf{Y}(t) = \begin{pmatrix} y_1(t) \\ y_2(t) \\ \cdots \\ \ldots \\ y_n(t) \end{pmatrix}$ is a vector of length *n* that is the measured UV absorption spectrum obtained at time t, $\mathbf{X} = \begin{pmatrix} 1 & x_{11} & \cdots & \cdots & x_{1p} \\ 1 & x_{21} & \cdots & \cdots & x_{2p} \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \ldots & \cdots & \ldots & \ldots & \cdots \\ 1 & x_{n1} & \ldots & \ldots & x_{np} \end{pmatrix}$ is the $n \times p+1$ design matrix where each p column corresponds to a spectrum of one of the compound that may be present in solution, $\beta(t) = \begin{pmatrix} \alpha(t) \\ ß_1(t) \\ \cdots \\ \ldots \\ ß_p(t) \end{pmatrix}$ is the slope vector of length p+1 and $\varepsilon(t)$ is the error vector. In this equation **Y** and **X** are known and the goal is to estimate $\beta(t)$ as each slope parameters in this vector correspond to the relative contribution of the corresponding spectrum in X to the measured Y spectrum. The slope parameters are directly related to the concentration in solution. This equation is a multi-linear regression (MLR) that can be solved using either matrix algebra or by minimizing the sum squared residuals (*SSR*) which is defined as

$$SSR = \sqrt{\sum_i (y_i - \hat{y}_i)^2} \quad (2)$$

where y_i and $\hat{y}_i$ are the true values and the estimated values respectively. However, for the solutions to be unique and accurate the following conditions should be verified: *i*. the spectrum of every compound that will contribute to the recorded spectrum, **Y**, should be taken into account in **X**; *ii*. each of those spectrum

78 should be independent (e.g. no multi-collinearity where one spectrum in **X** can be expressed as a linear
79 combination of other spectra) and *iii*. the number of column *p* (number of spectrum used in **X**) in the
80 design matrix should be much lower than the amount *n* of observation to avoid over-fitting. While SD is
81 sometime used with UV-vis spectrometer [16], to our knowledge it has never been applied to
82 chromatographic data where the aim is the quantification of few compounds. This is surprising as, because
83 of the separation mechanism, not only at every time few components should contribute to the recorded
84 spectrum, but the only spectra necessary are the spectra of the target compounds, the spectrum of the
85 background and the spectra of potential co-eluting species if those are different from the compounds of
86 interest. The description of the rest of the data is irrelevant for the quantification of the target peaks. It
87 should be emphasis that the aim in this work is the quantification of only some of the peaks for which
88 standard solutions are available. If all peaks are of interest or if standards are not available, multivariate
89 approaches should be preferred. Those approaches are a generalization of MLR applied to more than one
90 spectrum. They integrate statistical tools such as principal component analysis (PCA) to estimate the
91 design matrix. Numerous reviews in this subject are available [2,10,12,18-26].

92 The goal of this work was the quantification of diterpene esters in Arabica coffee brew by HPLC-
93 DAD. Diterpene are present in Arabica coffee in the form of cafestol and kahweol. Those compounds are
94 of interest due to their potential beneficial effect on human health. Literature surveys point out the anti-
95 carcinogenic, anti-inflammatory as well as anti-angiogenic properties of diterpenes [27,28]. In coffee,
96 diterpenes are rarely present in free form but as esters of fatty acids mainly: palmitic, linoleic, oleic and
97 stearic acids [29]. For their quantification by HPLC, cafestol and kahweol esters are normally converted
98 to their corresponding diterpene alcohols by a saponification reaction before being analyzed [30].
99 However, to study the health effect of those compounds, the quantification of the esterified form may
100 provide a better understanding. Kurzrock and Speer used GPC with LC-DAD-MS to identify different
101 kahweol and cafestol fatty esters in Arabica coffee [31]. However, some compounds were not baseline

separated could only be analyzed because of the MS. However they also shown that the kahweol esters could be selectively detected with the wavelength set at 290 nm but not the cafestol esters.

In this work, in absence of a MS detector, a mathematical separation using spectral deconvolution will be used in conjunction with LC-DAD (LC-DAD/SD) to obtain deconvolved chromatograms specific to the cafestol and kahweol esters. Because kahweol esters can be quantified using the chromatogram obtained at 290 or using deconvoluted chromatograms, those compounds will be used to compare the analytical performances of both methods. The eight diterpene esters of interest for this work are shown on table 1.

2. Materials and methods

2.1. Chemicals and samples

Cafestol linoleate, oleate and stearate along with kahweol linoleate, oleate and stearate, were obtained from LKT lab (MN, USA). Individual standards of cafestol palmitate and kahweol palmitate were acquired from Sigma–Aldrich (MO, USA). Acetonitrile, methanol (HPLC gradient grade) and diethyl ether were obtained from VWR (Belgium). Potassium hydroxide powder and sodium chloride were supplied by Merck (Germany) and Panreac Quimica (Spain), respectively. All stock were prepared in acetonitrile with the following concentration: cafestol and kahweol palmitate (300 mg/L), cafestol and kahweol linoleate, oleate, stearate (200 mg/L). To protect standards from degradation under sun light or heat, all of them were wrapped in aluminum foil and stored in -22 ºC.

2.2. Separation and detection

Coffee brews were prepared by boiling ground coffees (11.25 g of pure Arabica) with 150 mL of distilled water for 10 min followed by 2 min of settling time followed by decanting the liquid. Three cups (150 mL) were prepared and kept in -22 ºC. Prior to the extraction, frozen samples were defrosted and

125 mixed, heated and stirred well to reach a homogeneous mixture at 55-60 ºC. Extraction of diterpene esters
126 were performed in duplicate according to the developed and validated methodology described previously
127 by Moeenfard *et al.* [32]. Briefly, 2.5 mL of coffee brew were extracted directly using 5 mL of diethyl
128 ether. The mixture was vortexed for 2 min and after centrifugation (Rotofix 32A, Germany) at 4000 rpm
129 during 10 min, the upper phase was transferred to a clean test tube. The aqueous solution was re-extracted
130 using the diethyl ether then the combined ether phase was washed with 5 mL of 2M NaCl solution to
131 remove interfering compounds followed by centrifugation (4000 rpm, 10 min). In each step of extraction
132 and cleaning, 0.5 mL of methanol was added to break the emulsion and create a neat interface between
133 aqueous and organic phases. The clean ether phase was transferred to an amber glass dried under N_2
134 stream. Samples were kept in -22 ºC until analysis using LC-DAD.
135 Analysis was carried out in a Merck Hitachi Elite LaChrom (Tokyo, Japan) system equipped with a
136 quaternary pump (L-2130), an L-2200 autosampler. Separation was achieved using a Purospher STAR
137 LichroCART RP 18 end-capped (250 × 4 mm, 5 µm) column attached to a guard column (4 × 4 mm, 5µm)
138 of the same kind. Prior to injection, dried extract was dissolved in 2.5 mL of acetonitrile and filtered
139 through 0.45 µm filter membrane (PTFE, VWR, USA). The chromatographic conditions for analysing
140 diterpenes esters were adapted from [33] with slight modifications. Twenty microliter of sample was
141 injected and the separation was achieved using isocratic conditions during 30 min whith the mobile phase
142 made of acetonitrile/isopropanol (70/30, v/v) and pumped at 0.4 mL/min. The detection was made using
143 through L-2455 (Merck Hitachi) UV/vis spectrophotometry diode array detector in the range of 200 to
144 400 nm. Two detection wavelengths were also set: 225 nm and 290 nm for cafestol esters and kahweol
145 esters respectively. EZChrom Elite 3.1.6 software was used for data acquisition and analysis.

146 *2.3. Deconvolution*

147 After each run, data were exported as coma separated values (CSV) format by the acquisition software
148 (EZChrom Elite 3.1.6). Those files were open using Matlab R2013b and the chromatographic data loaded

into a two-dimensional array. The different Matlab functions were programmed using Matlab 2013b and run on a personal computer equipped with 4GB of RAM. The code and procedures used are further described in supplementary information (SI).

3. Results and discussion

3.1. Separation and selection of the model

Different mobile phase compositions and flow rates were assayed using HPLC-DAD to separate the target compounds, namely: acetonitrile/isopropanol (70/30) at 0.2, 0.4 and 0.8 mL/min; acetonitrile/isopropanol (90/10) at 0.4, 0.6 and 0.8 mL/min; acetonitrile/isopropanol (50/50) at 0.4 and 0.6 mL/min; acetonitrile/methanol (70/30) at 0.8 mL/min; acetonitrile/methanol (90/10) at 0.8 mL/min. Results were not better than in a previously published work [33]. Within our experimental trials, the best separation was obtained using acetonitrile/isopropanol (70/30, v/v) with a flow rate equal to 0.4 mL/min. Chromatograms of a coffee extracted and separated as described in material and methoda are presented in Fig. 1, with the wavelength set at (A) 205 ±4 nm, (B) 225 ±4 nm and (C) 290 ±4 nm. The peaks corresponding to the eight diterpene esters of interest are indicated by numbers (see table 1). While, all kahweol esters are baseline separated and can be quantified setting the wavelength to 290 nm (Fig. 1C) this is not the case for the cafestol esters. For those compounds there is no wavelength that is specific enough to resolve the coelution problem between the cafestol and kahweol esters. As the separation of those compounds is particular difficult and in absence of a MS detector a mathematical separation may be the easiest approach. Because the kahweol esters can be quantified using a classical method, those compounds will be used to compare the analytical performances of both approaches.

The first step in the spectral deconvolution is to build the design matrix, **X** (see eq. 1), that will contain all the spectra of the compounds of interest, in this case the 8 diterpene esters, a background spectrum and a constant as well as the spectra of potential interfering compounds. To obtain reliable spectra a series of samples, each of them containing one diterpene ester a concentration of 100 mg/L, were injected and

separated. The spectrum for each diterpene ester was measured using the peak of the main compound and the spectrum was corrected for background adsorption (see supplementary information for more details). The resulting spectra are presented in Fig. 2, with (A) the four spectra related to the kahweol esters, (B) the four spectra related to the cafestol esters, (C) a background spectrum and (D) the spectra of potential interfering species. All spectra have been normalized by their highest absorbance value to facilitate their visual comparison. As it can be seen spectra from the same diterpene only differ by their absorbance values below 220 nm. The matrix of correlation between the different spectra was calculated using Matlab. All the kahweol esters were found to be correlated with a score higher than 0.93 and all the cafestol esters with a score higher than 0.91. Because the background also absorbs below 220 nm, the data below 220 nm were removed as well as the data over 320 nm, this to (1) avoid problem of colinearity with the background spectra and (2) remove the range where the diterpene esters do not absorbed. Within this new wavelength range, spectra from the same diterpene correlated with a score higher than 0.998 and the same spectra were used for the four cafestol esters and the four kahweol esters. Two deconvolution models will be used subsequently, the 4-spectra model (one spectrum for all the cafestol esters, one spectrum for all kahweol esters, one background spectrum and one constant in the design matrix) and the 7-spectra model that is the same spectra as previously plus three spectra from impurities that may co-elute with the diterpene esters and that were detected in the standards. Those three additional spectra are shown in fig. 2D.

3.2. Spectral deconvolution

Using Matlab, SD was applied to every spectrum acquired during the separation (see SI). Deconvolved chromatograms were obtained as the variation as a function of time of one of the slope parameter obtained by solving eq. 1. As an example, Fig. 3 shows the deconvolved chromatograms in respect to the kahweol (A) and cafestol (B) spectra obtained with the 4-spectra deconvolution model (I) or the 7-spectra deconvolution model (II). Fig. 3 (IC) and (IIC) are the plot of the *SSR* as a function of

197 time. Those chromatograms correspond to the separation of a sample made by mixing the eight standards
198 of diterpene esters at equal concentration (50 mg/mL). As it can be seen both model were successful in
199 separating the contribution from kahweol and cafestol from the original data. The main differences
200 between the 4- and 7-spectra models are in the minor peaks. This is particularly noticeable comparing the
201 peak of KS (peak 4) in fig 3 (IB) and fig 3 (IIB) or comparing the *SSR* (fig 3 (IC) and fig 3 (IIC)).
202 Deconvolved chromatograms will be used subsequently in the same way as chromatogram to quantify our
203 target chemicals.

204 *3.3. Analytical performances*

205 The analytical performances obtained when quantifying the kahweol esters with a classical approach
206 with detection at 290 nm or after SD with a 4-spectra model and a 7-spectra model were compared, this
207 to validate the HPLCE-DAD/SD methodology. In the deconvolution model, the same spectra were always
208 used in the exception of the background spectra which was measured in each experiment.
209 The calibration curves were build using standard solutions, each of them containing the 8 diterpenes
210 esters at known concentration (concentrating ranging from 2 to 600 mg/L for CP and KP (2, 5, 10, 20, 50,
211 150, 300 and 600 mg/L) and between 2 to 600 mg/L (2, 5, 10, 20, 50, 100, 200 and 600 mg/L) for CO,
212 CL, CS, KO, KL and KS). In the classical approach the peak areas were measured using the acquisition
213 software. With the deconvolved chromatograms peak areas were measured using a small routine
214 programed in Matlab (see SI). Each sample solution was run in duplicate. Results obtained are presented
215 in Table 2. Slope, intercept, r^2, standard error and the random errors in the *y*-direction ($\sigma_{y/x}$) were
216 calculated using the LINEST function from Excel. *LODs* were calculated as the concentration that will
217 give a signal equal to the intercept plus three times $\sigma_{y/x}$ [34]. As it can be seen in this table, the three
218 approaches present similar performances with very good linearity. Comparing the *LOD*s, best results were
219 obtained using the 7-spectra deconvolution model (average 5.7 mg/L; min KO, 5.4 mg/L; max KP, 6.2
220 mg/L), followed by the classical approach (average 6.7 mg/L; min KO, 4.9 mg/L; max KS, 9.0 mg/L) and

221 the 4-spectra deconvolution model (average 7.1 mg/L; min KP, 4.8 mg/L; max KL, 10.8 mg/L). A similar
222 result was obtained with the cafestol diesters with an average *LOD* using the 7-spectral model of 2.2 mg/L
223 (min CL, 1.0 mg/L; max CS, 3.4 mg/L) and of 3.2 mg/L (min CL, 1.6 mg/L; max CS, 6.2 mg/L) for the
224 4-spectra model, clearly demonstrating that the 7-spectra model gives better results.
225 The intra-day repeatability was measured using the 7-spectra model with six successive runs using a
226 mixture of the eight diterpene esters, each at a concentration of 75 mg/L. The average intra-day peak area
227 *RSD* was measured equal to 0.8 % (min CL, 0.3%; max KO, 1.7%). The inter-day repeatability was
228 measured in 3 successive days, each sample run in triplicates. The average inter-day peak area *RSD* was
229 measured equal to 1.0 % (min KL and KS, 0.5%; max CS, 1.4%).

230 *3.4. Application to a real coffee sample.*

231 After extraction as detailed in experimental, a coffee brew (boiled coffee prepared using 100%
232 Arabica coffee) was separated and the concentration of the 8 diterpenes esters was measured. Three
233 extractions were done, each extraction run in duplicates. Deconvolved chromatogram can be seen in fig
234 5 with (A) the deconvolved chromatogram related to cafestol, (B) the deconvolved chromatogram related
235 to kahweol and (C) the plot of the relative *SSR*. Results obtained using the chromatogram at 290 nm (only
236 for KL, KO, KP, KS) or using the deconvolved chromatograms are resumed in table 3. In this table, the
237 *t*-test (two-tail) between the two series was calculated using Excel. A value of *P* higher than 0.05 indicates
238 that the values are not significantly different [34], this is the case for all calculated concentration of
239 kahweol esters indicating perfect agreement between the two approaches. Cafestol could only be
240 calculated after spectral deconvolution. It should be noted that the *SSR* shows in fig 4C are, at least, one
241 order of magnitude higher than when working with the mixture of standard (fig 3(IC) and 3(IIC)). This is
242 particularly noticeable for KO, KP, CO and CP. This is probably due to the presence of co-eluting species
243 whose spectra are not in in the model. This is not surprising when working with complex matrix and real
244 samples. A better precision could probably be obtained if the spectra of those impurities were taken into

account in the design matrix. However, in this particular example the error is small and should not significantly contributed to the measured concentration in table 3. This is also validated by the excellent agreement obtained using both approaches as demonstrated in table 3. It should also be emphasis that such problems also occur with a classical chromatogram. However it is generally unnoticed. Here the *SSR* allows to verify the goodness of the model and to detect the presence of co-eluting species even with perfect coelution, this as long as the spectra of the impurity is significantly different from the one of the main components. It should also be noted in fig. 4C the very high *SSR* of the peaks marked by an asterisk. Those peaks are due to unknown components that are not taking into account in the model. It is evident that those peaks will not impact the quantification of the target compounds and can be ignored.

4. Conclusions

LC-DAD/SD has been successfully applied to deconvolve, from the raw data, the contribution from cafestol and kahweol esters. In case of kahweol esters that could be quantified using the deconvolved chromatogram and a chromatogram obtained at 290 nm, slightly better analytical performances were obtained using the deconvolved chromatogram. However LC-DAD/SD demonstrated its full potential with the analysis of cafestol esters that could not have be achieved otherwise. This approach was demonstrated to be accurate and cheap.

However LC-DAD/SD is not a universal solution. While mathematical deconvolution could be an integrate part of the analytical method when using DAD, it should also be rigorously designed and validated. In particular, it is important to obtain high quality spectra from standards and to verify for collinearity. The amount of spectra used at any time should also be kept minimal. While, in theory only the spectra of the target compounds and background spectra are needed this is with the assumption that no impurities are co-eluting. If this is not the case and if the concentration of the impurities are high enough to interfere, the spectra of the impurities should be added in the design matrix. A careful

examination of the plot of the *SSR* allow visually to assess the performance of the deconvolution model and to optimize it if necessary.

Acknowledgments

TO BE DONE.

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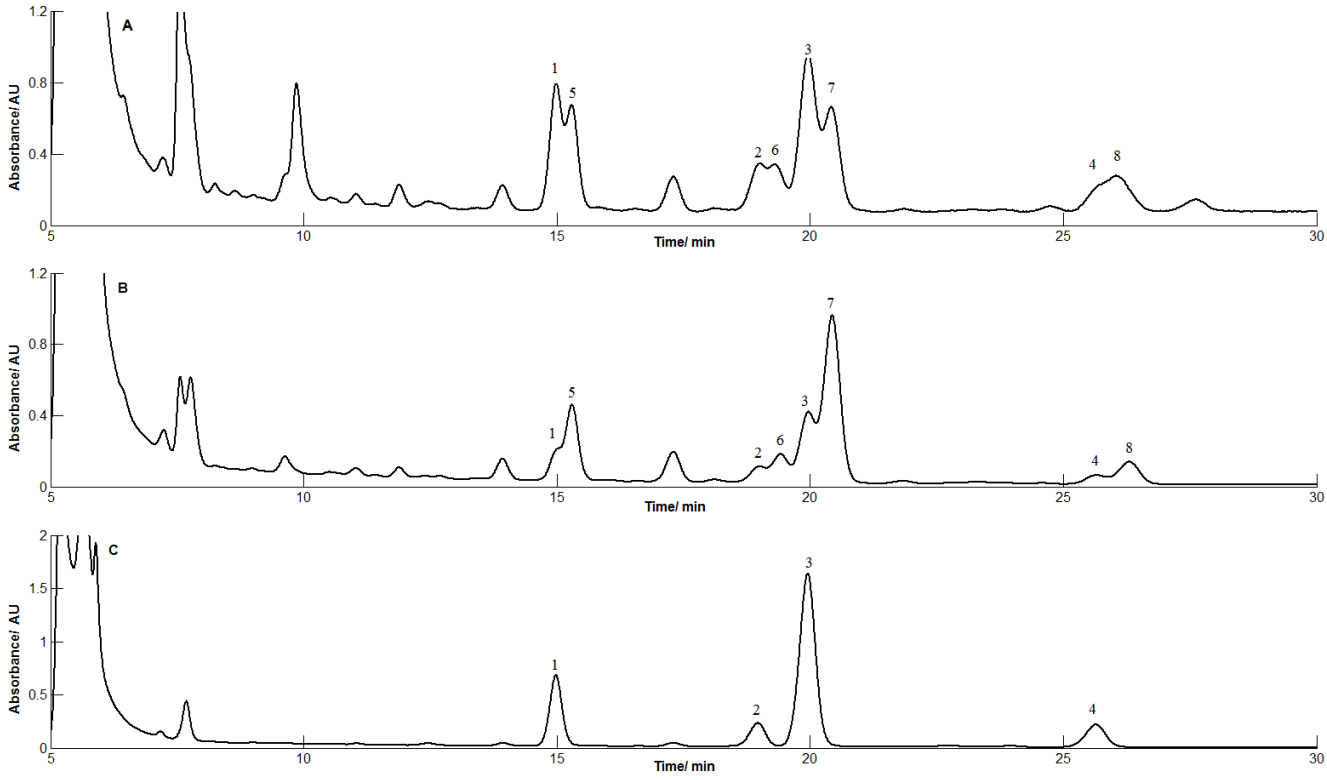


325

326 **Figure 1**. Separation of diterpene esters in coffee by LC-DAD with detection at (A) 205 ± 4 nm, (B)
327 225 ± 4 nm and (C) 290 ± 4 nm. The coffee sample (boiled coffee produced with 100% Arabica coffee)
328 has been extracted with diethyl ether and separated using a C18 column with a mobile phase constituted
329 of acetonitrile/isopropanol (70/30, v/v) at a flow rate of 0.4 mL/min. The peaks corresponding to the
330 diterpene esters are indicated as (1) (KL: Kahweol linoleate), (2) (KO: Kahweol oleate), (3) (KP: Kahweol

palmitate), (4) (KS: Kahweol stearate), (5) (CL: Cafestol linoleate), (6) (CO: Cafestol oleate), (7) (CP: Cafestol palmitate), and (8) (CS: Cafestol stearate).

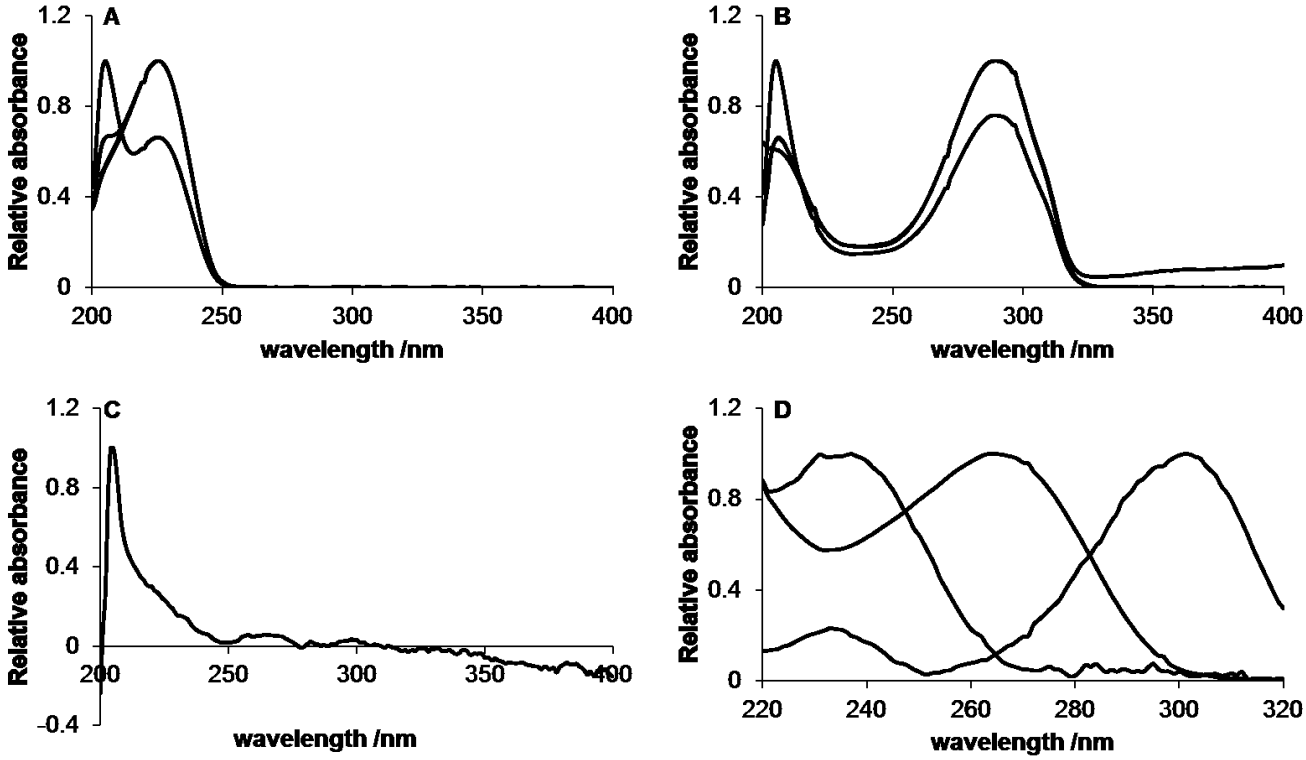
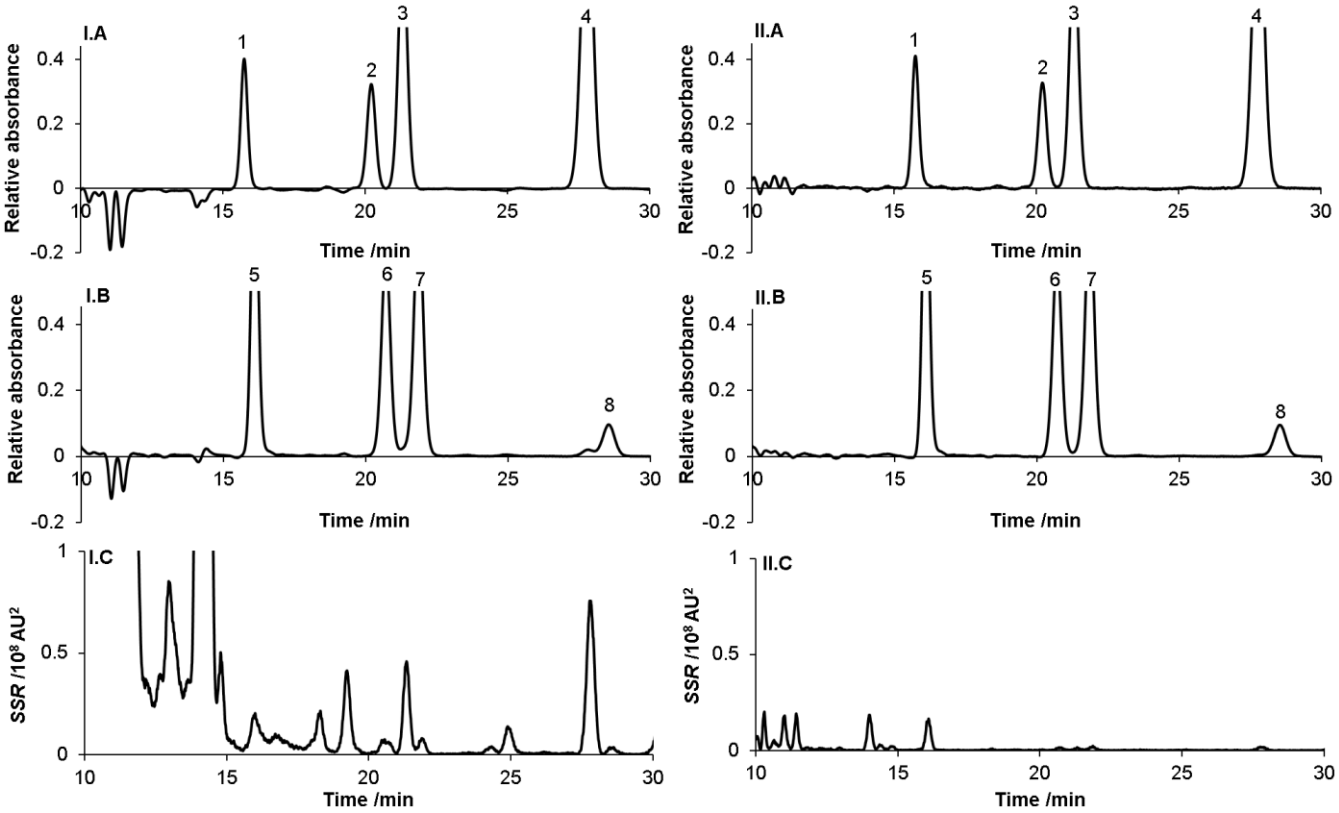


Figure 2. UV-vis absorption spectra of (A) cafestol esters, (B) kahweol esters, (C) background and (D) main impurities.



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339 **Figure 3.** Deconvolved chromatograms obtained using LC-DAD/SD with of a standard mixture of
340 diterpene esters. Panels with the suffix I display chromatogram obtained with a 4-spectra model and Panel
341 with the suffix II display chromatogram obtained with a 7-spectra model. Panels with the suffix A, B and
342 C display the deconvolved chromatogram related to kahweol, the deconvolved chromatogram related to
343 cafestol and the sum squared residuals respectively. Other conditions as in figure 1.

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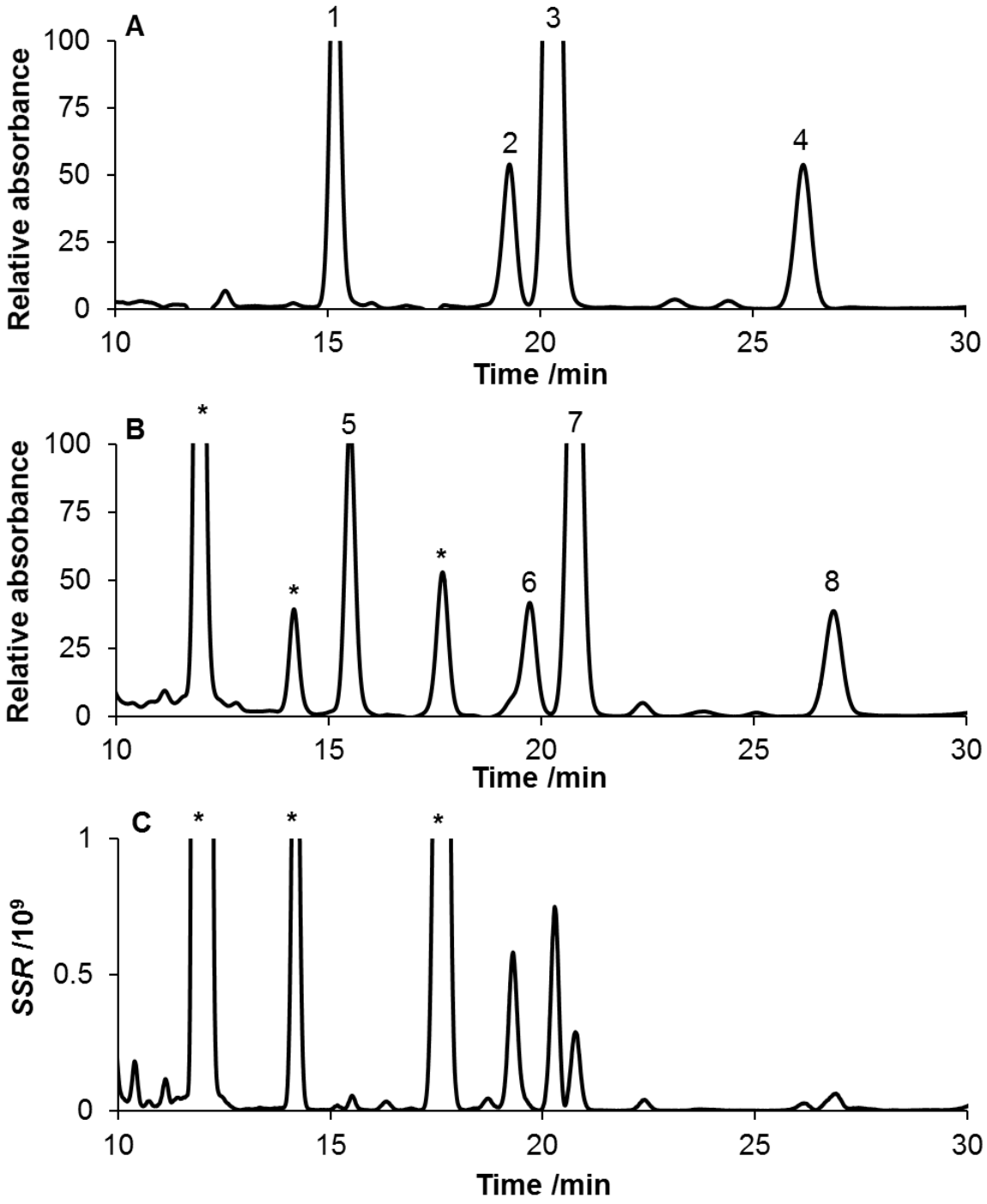


345

346 **Figure 4.** Separation of diterpene esters in a coffee sample (boiled coffee), performed by LC-
347 DAD/SD. (A), (B) and (C) are the deconvolved chromatogram related to the cafestol spectrum, the
348 deconvoluted chromatogram related to the cafestol spectrum and the plot of the relative sum square
349 residual respectively. Peaks marked with an asterisk indicate the position of main impurities. Other
350 conditions as in figure 1

351

Table 1. Names and structures of the diterpenes esters of interest.

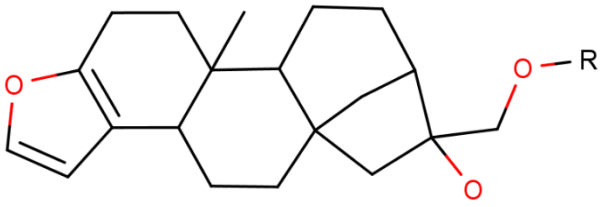
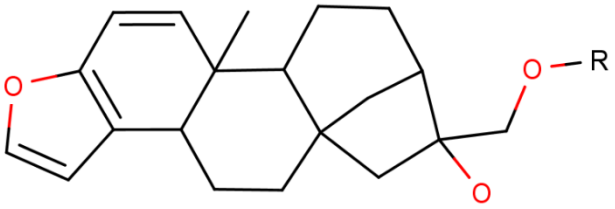
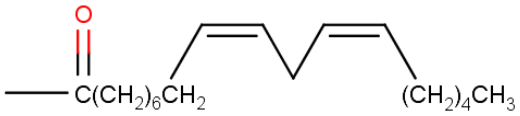
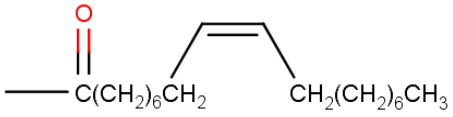
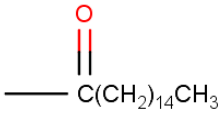
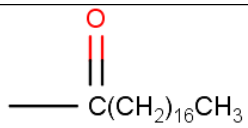
 **Cafestol esters**	 **Kahweol etsers**
with side chain R as:	
	Cafestol – linoleate (CL) Kahweol – linoleate (KL)
	Cafestol – oleate (CO) Kahweol – oleate (KO)
	Cafestol – palmitate (CP) Kahweol – palmitate (KP)
	Cafestol – stearate (CS) Kahweol – stearate (KS)

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Table 2. Statistic for the calibration curves of KL (Kahweol linoleate), KO (Kahweol oleate), KP (Kahweol palmitate), and KS (Kahweol stearate) obtain using Excel (LINEST function).

	Spectral Deconvolution								**Classical**			
	4-spectra model				7-spectra model				290 ± 4 nm			
Compound	KL	KO	KP	KS	KL	KO	KP	KS	KL	KO	KP	KS
Slope[a]	162 (±2)[c]	169 (±2)[c]	356 (±9)[c]	643 (±4)[c]	429 (±3)[c]	394 (±3)[c]	1017 (±5)[c]	1727 (±13)[c]	(25.3(±0.02)[c]) x10^3	(23.5(±0.01)[c]) x10^3	(56.1(±1.1)[c]) x10^3	(104.8(±0.8)[c] x10^3
Intercept[a]	-283 (±227)[c]	-224 (±194)[c]	546 (±239)[c]	-370 (±400)[c]	-121 (±286)[c]	41 (±243)[c]	-218 (±696)[c]	-547 (±1116)[c]	(-1.0(±2.0)[c]) x10^4	(-0.2(±1.5)[c]) x10^4	(7.9(±3.0)[c]) x10^4	(--6.2(±7.6)[c]) x10^5
r^2	0.9978	0.9985	0.9963	0.9996	0.9993	0.9994	0.9997	0.9993	0.9993	0.9995	0.9976	0.9994
$\sigma_{y/x}$[a]	582	498	462	1022	828	703	2088	3225	52133	38479	58800	194900
LOD[b] (mg/L)	**10.8**	**8.8**	**3.9**	**4.8**	**5.8**	**5.4**	**6.2**	**5.6**	**6.2**	**4.9**	**3.1**	**5.6**

[a] Values calculated by excel using the LINEST function

[b] The *LOD* is calculated as the concentration at which the amplitude of the response is equal to the intercept plus three time $\sigma_{y/x}$.

[c] Value in bracket are the standard deviations.

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Table 3. Concentration of cafestol and kawheol esters in a coffee sample.

	Cafestol				**Kawheol**			
	CL	CO	CP	CS	KL	KO	KP	KS
Classical (290 ±4 nm)	*NA*	*NA*	*NA*	*NA*	448.4 (±8.6)[b]	209.7 (±5.1)[b]	594.6 (±13.9)[b]	57.5 (±1.9)[b]
Deconvolution (7-spectra model)	57.9 (±1.1)[b]	37.2 (±1.9)[b]	160.2 (±4.9)[b]	202.4 (±14.3)[b]	447.4 (±8.9)[b]	204.2 (±7.4)[b]	599.7 (±13.7)[b]	57.0 (±2.1)[b]
P(T ≤ t)[a]	*NA*	*NA*	*NA*	*NA*	0.86	0.22	0.58	0.68

[a] t-test for two samples calculated by Excel. P(T ≤ t) > 0.05 indicates that the values are not significantly different.

[b] Values in bracket are the standard deviations.

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