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Novel combination of non-aqueous capillary electrophoresis and multivariate curve resolution-alternating least squares to determine phenolic acids in virgin olive oil☆

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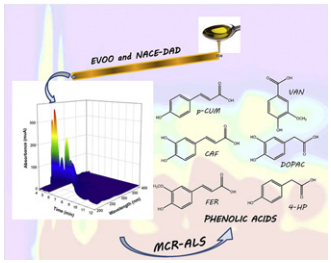
HIGHLIGHTS

- Novel combination of NACE and MCR-ALS for determination of phenolic acids in EVOO.
- Good results are achieved in less time than other CE method for these compounds.
- Resolution and quantitation without to be necessary a complex experimental work.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper presents the development of a non-aqueous capillary electrophoresis method coupled to UV detection combined with multivariate curve resolution-alternating least-squares (MCR-ALS) to carry out the resolution and quantitation of a mixture of six phenolic acids in virgin olive oil samples. *p*-Coumaric, caffeic, ferulic, 3,4-dihydroxyphenylacetic, vanillic and 4-hydroxyphenilacetic acids have been the analytes under study. All of them present different absorption spectra and overlapped time profiles with the olive oil matrix interferences and between them. The modeling strategy involves the building of a single MCR-ALS model composed of matrices augmented in the temporal mode, namely spectra remain invariant while time profiles may change from sample to sample. So MCR-ALS was used to cope with the coeluting interferences, on accounting the second order advantage inherent to this algorithm which, in addition, is able to handle data sets deviating from trilinearity, like the data herein analyzed. The method was firstly applied to resolve standard mixtures of the analytes randomly prepared in 1-propanol and, secondly, in real virgin olive oil samples, getting recovery values near to 100% in all cases. The importance and novelty of this methodology relies on the combination of non-aqueous capillary electrophoresis second-order data and MCR-ALS algorithm which allows performing the resolution of these compounds simplifying the previous sample pretreatment stages.

Abbreviations: VOO, virgin olive oil; NACE, non-aqueous capillary electrophoresis; CZE, capillary zone electrophoresis; BGE, background electrolyte; p-CUM, *p*-coumaric acid; CAF, caffeic acid; FER, ferulic acid; DOPAC, 3,4-dihydroxyphenylcacetic acid; VAN, vanillic acid; 4HP4, -hydroxyphenylaceitc acid; LOD, limit of detection; LOQ, limit of quantitation.

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1. Introduction

Under the denomination "phenolic compounds" there are more than 4000 compounds divided in 12 subclasses [1]. Currently, these compounds are receiving considerable attention, fundamentally due to its antioxidant activity, strongly related to the prevention of cancer, inflammatory disorders and cardiovascular diseases [2,3]. They are part of the minor components of virgin olive oil (VOO), one of the most important foods in the Mediterranean diet which has associated many benefits for the human health, essentially due to its content in these compounds [4]. In addition, phenolic compounds and their strong natural antioxidant activity contribute to the stability of VOO against oxidation and influence in its organoleptic characteristics and nutritional qualities [5]. The composition of phenolic compounds in VOO is related to agronomic and technological aspects [6].

For the quantitation of phenolic compounds in VOO it is important to carry out a complete extraction of this fraction from the oil. Table 1 shows the most used procedures (both traditionally and nowadays) and a comparison between them. Both the liquid–liquid extraction (LLE) and the solid phase extraction (SPE) procedures are complex, tedious and time consuming. In addition, it is habitual to have a great consumption of toxic solvents, like hexane [1]. Nowadays, the traditional methods for detection and quantitation of phenolic compounds have been replaced by separation techniques, such as gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) coupled to different detectors [12–15,20,21]. CE is getting importance and popularity for the analysis of food components, mainly due to the combination of short analysis time and high separation efficiency [22]. In addition, and especially in the case of non-aqueous matrices as those of olive oil samples, the pretreatment of the sample can be greatly simplified using the non-aqueous capillary electrophoresis (NACE) mode. Compared with aqueous capillary zone electrophoresis (CZE), NACE has the advantages of wide bore capillary as a consequence of a minor Joule effect, fast analysis since it is possible to use a higher separation voltage, low adsorption on the capillary wall, and high separation selectivity by selecting the adequate non aqueous background electrolyte (BGE) [23].

Ideally, in optimized conditions, electrophoretic experiments lead to total analytes separation, i.e. each peak belongs to a single compound. It is interesting to note that, although a complete separation of the peaks could not be performed, second order data coupled to chemometrics can be used to achieve selectivity by mathematical means, allowing for resolution and quantitation of overlapped analytes [24,25]. The information provided by the second-order signals, adequately decomposed by suitable second-order algorithms, can be uniquely ascribed to the analyte of interest, even in the presence of unexpected components not considered in the calibration stage. This property is called the second-order advantage and avoids the requirement of physically removing interferences [26,27]. Among the available second order algorithms, MCR-ALS and PARAFAC2, a variant of PARAFAC (parallel factor analysis) [28], are those able to handle second order data deviating from trilinearity, i.e. when changes in shape and/or position of component profiles from sample to sample occur, which is commonly found in capillary electrophoresis data [29–32]. To overcome this challenge, MCR-ALS was performed in the so-called extended mode [33], which involves building an augmented data matrix by appending calibration and test data matrices in the time direction, i.e. the rows represent spectra and the columns time profiles, because this alleviates the problems associated with sample-to-sample differences in this dimension.

Regarding the published works in this context, Sentellas and Saurina reviewed in 2003 the application of chemometrics in CE, in which the methods for data analysis [34] and optimization [35] were introduced. In later years, both first- and second-order methods have been also used for quantification in CE, including principal component regression (PCR), partial least squares regression (PLS), multiple linear regression (MLR), artificial neural networks (ANN) [36–38] and MCR-ALS [24,32,39]. CE coupled with chemometric methods enhances its ability of separation and analysis tremendously. Regarding the combination of chemometric and CE for food analysis, many papers have been published in the authentication and characterization field [36,37]. However, to the best of our knowledge, no paper has been published regarding the use of second order data and CE in the food analysis field for resolution and quantitation. In this context, we pretend innovatively to develop a non-aqueous capillary electrophoresis method coupled to UV detection (NACE-DAD) and combine it with the MCR-ALS algorithm to carry out the resolution and quantitation of a complex mixture of six phenolic acids in VOO samples, in a short period of time and without being necessary a complex experimental work.

2. Theory

2.1. Baseline correction adapted to second order data

Generally, the elimination of baseline is crucial for reducing the number and complexity of the unexpected components. In this work, baseline correction was carried out according to the asymmetric least-squares methodology proposed by Eilers [40] and adapted to second-order data [41], which consists in the minimization of the cost function:

$$Q = \sum_i v_i (y_i - f_i)^2 + \lambda \sum_i (\Delta^2 f_i)^2 \quad (1)$$

in which y is the experimental signal, f is a smooth trend (the baseline approximation), and v is a prior weight. The elements of v are 1 in all places where y is observed or allowed to influence f, while, in all other places, these elements are 0. The positive parameter λ sets the second term weight. It acts as a roughness penalty: the larger λ, the smoother f will be. Δ denotes the derivative of f.

Taking into account the following choice of asymmetric weights: $v_{JK} = p$ if $y_{JK} > f_{JK}$ and $v_{JK} = 1 - p$ if $y_{JK} \leq f_{JK}$ with $0 < p < 1$, positive deviation from the trend will get weights different from negative residuals. Experience demonstrates that a quick and reliable solution could be achieved in about 10 iterations, starting from $v \cong 1$ and iterating between the two computations.

2.2. MCR-ALS

MCR-ALS is an algorithm capable of handling data sets deviating from trilinearity, i.e. data in which migration time shifts or peak shape changes occur for analytes from sample to sample. This can be done due to the strategy of augmenting matrices along the mode which is suspected of breaking the trilinear structure, i.e. if matrix-to-matrix variation of profiles occurs along the column direction, a column-wise augmented matrix is created. The bilinear decomposition of the augmented matrix **D** is performed according to the expression:

$$\mathbf{D} = \mathbf{C} \times \mathbf{S}^{\mathbf{T}} + \mathbf{E} \quad (2)$$

in which the rows of **D** contain the UV–Vis spectra (K wavelengths), as a function of time (J times), the columns of **C** contain the time profiles of the N compounds involved in the process, the columns of **S** their related spectra, and **E** is a matrix of residuals not fitted by the model. Decomposition of **D** is achieved by iterative least-squares minimization of $||\mathbf{E}||$, under suitable constraining conditions, i.e. non-negativity in the spectral profiles, unimodality and non-negativity in the time profiles, correspondence among