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Matrix solid-phase dispersion: a simple and fast technique for the determination of phenolic compounds in olive oil by liquid chromatography

Romina P. Monasterio,*[ab] Ariel R. Fontana[a] and María Fernanda Silva[a]

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A methodology was developed using matrix solid-phase dispersion, together with liquid chromatography with multiple wavelength detectors for the determination of 20 phenolic compounds in olive oil samples. Under optimized conditions, the analytes were extracted using 0.5 g of olive oil, 1.0 g of Florisil as a sorbent and 1 mL methanol–water (80 : 20 acidified with formic acid 0.5% (v/v)) as an eluting solvent. The proposed methodology provided detection and quantification limits of individual compounds in the ranges of 0.02–0.75 and 0.08–2.50 mg kg^{-1}, respectively. The RSDs resulting from the analysis of six replicates of 0.5 g of a sample pool containing 2.5 mg kg^{-1} phenolic compounds were ranged between 2.1% and 14.8%. Considering matrix-matched calibration as a quantification technique, the average recoveries ranged from 74.8% to 95.0% with relative standard deviations between 1.5% and 9.3%. The developed methodology was applied for the determination of phenolic compounds in nine olive oils produced in Argentina, identifying seventeen analytes at concentrations above the detectable levels.

1. Introduction

Virgin olive oil is extracted from the olive fruit (*Olea europaea* L.) solely mechanically without further treatment other than washing, filtration, decantation, or centrifugation.[1] It is almost unique among vegetable oils because it can be consumed without any refining treatment. The absence of a refining process allows the presence of minor biomolecules, such as vitamins, carotenoids, tocopherols, phenolic compounds, and other natural antioxidants, which may act, by different mechanisms, as an effective defense against reactive oxygen substances.[2–4] These minor biomolecules are present in about 2% of nearly 250 components; the remaining 98% mainly consist of triacylglycerols.[5,6]

Phenolic compounds are an important group of natural compounds that contribute to flavor, color, and secondary properties such as bitterness and astringency.[6,7] Owing to the complexity of sample matrices and the low concentration of phenolic compounds, it is difficult to directly determine these compounds in olive oil. Hence, sample preparation becomes a crucial step in the accurate and sensitive determination of these analytes. The most commonly reported technique for the separation and pre-concentration of different compounds in olive oil are based on liquid–liquid extraction (LLE) with solvents of different polarities,[8,9] gel permeation chromatography (GPC),[10] dispersive solid-phase extraction (DSPE)[11] and solid-phase extraction (SPE).[12] Nevertheless, synchronous with modern trends in analytical chemistry towards the simplification and miniaturization of sample preparation techniques, some modifications must be considered. Some disadvantages, such as large volumes of toxic and expensive solvents, high amount of wastes and reduced frequency of analysis, can effectively overcome. In this manner, solid-phase microextraction (SPME),[13] quick, easy, low-cost, effective, rugged and safe (QuEChERS) techniques[14] or matrix solid-phase dispersion (MSPD)[15] have been more employed.

The MSPD can combine the steps of homogenization, extraction and purification into one procedure and has been proven to be an effective technique for sample pretreatment ranging from solid to semi-solid and highly viscous samples.[16,17] MSPD has some major advantages such as straightforward application, ability to simultaneously perform extraction and cleanup in a single step with good recovery and precision.[16,18] This technique has been increasingly applied for the extraction of various compounds or classes of compounds from several complex matrices. Specifically, MSPD has been used for flavonoids in citrus fruit juice and human fluid samples,[17,19] the degradation products of organosulfur compounds used as fungicides in strawberries,[20] phenolic compounds in pickled quail eggs, green tea and wine[21,22] and pesticide determination in olives and olive oil,[15,23] prior to GC and HPLC determination.

[a]*Instituto de Biología Agrícola de Mendoza (IBAM-CONICET), Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Almirante Brown 500, Chacras de Coria, Mendoza, Argentina. E-mail: rmonasterio@mendoza-conicet.gob.ar; Fax: +54-261-4960469; Tel: +54-261-4135000 ext. 1307*

[b]*Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa, Argentina*

Although MSPD received a favorable response, the applicability of this technique for oil-based is few explored and particularly its application for the extraction of phenolic compounds from virgin olive oil has not been previously reported.

On the other hand, reversed-phase high-performance liquid chromatography (RP-HPLC) and diverse modes of capillary electrophoresis (CE), coupled with different detectors, such as UV-vis, multi-wavelength (MWD), fluorescence, electrochemical and mass spectrometry (MS), are the most widely employed analytical methods for detecting and quantifying phenolic compounds in oils.[2,24]

In the present work, MSPD was applied for the extraction of 20 phenolic compounds in olive oil samples prior to its determination by HPLC-MWD. Several sample pretreatment parameters, including the MSPD sorbent, the rinsing and eluting solvents, and the conditions for the separation and determination of the multiclass phenolic compounds by HPLC-MWD have been studied. The analytical performance was evaluated in terms of limits of detection (LODs), recoveries, precision and linear range. Finally, the developed methodology was applied for the determination of target phenolic compounds in olive oil samples from Argentina, in order to establish the robustness of MSPD-HPLC-MWD.

2. Experimental

2.1 Reagents and materials

Standards of caffeic (CAF), gallic (GAL), vanillic (VAN) ≥97.0% (Fluka, Buchs, Switzerland), syringic (Sy) ≥95% (Sigma-Aldrich, Milwaukee, WI, USA), *p*-coumaric (*p*-COU) ≥98.0% (Sigma-Aldrich), *trans*-ferulic (FER) ≥99% (Sigma-Aldrich), 4-hydroxyphenylacetic (HPH) 98% (Sigma-Aldrich), 2,5-dihydroxybenzoic (DHB) 98% (Sigma-Aldrich), sinapic (SIN) ≥98% (Sigma-Aldrich), chlorogenic (CHL) ≥95% (Sigma-Aldrich) acids, oleuropein (OLE) ≥80% (Sigma-Aldrich), apigenin (API) ≥95.0% (Sigma-Aldrich), luteolin (LUT) (Fluka), 3-hydroxytyrosol (HTY), pinoresinol (PIN) ≥99.5% (Sigma-Aldrich), quercetin 3-β-D-glucoside (QUE) ≥90% (Sigma-Aldrich), rutin (RUT) ≥94% (Sigma-Aldrich), kaempferol (KAE) ≥90% (Sigma-Aldrich), catechin (CAT) ≥98% (Sigma-Aldrich) and 2-(4-hydroxyphenyl)ethanol (tyrosol) (TY) ≥99.5% (Fluka) were used. The phenolic compound stock solutions were prepared by

Table 1 Conditions for the separation and detection parameters of targeted analytes

Flow gradient conditions

Time	Flow rate (mL min^{-1})	A% water with 0.1% formic acid (v/v)	B% methanol	Gradient
0	1	80	20	Linear
1	1	70	30	Linear
16	1	70	30	Linear
30	1	40	60	Linear
32	1	80	20	Linear
38	1	80	20	Linear

Detection parameters

No.	Analyte	Abbreviation	t_R (min)	Quantification λ^a (nm)
1	Gallic acid	GAL	2.9	280
2	3-Hydroxytyrosol	HTY	3.3	280
3	Catechin	CAT	4.0	280
4	Tyrosol	TY	4.4	280
5	4-Hydroxyphenylacetic acid	HPH	4.6	280
6	2,5-Dihydroxybenzoic acid	DHB	5.0	320
7	Chlorogenic acid	CHL	5.3	320
8	Caffeic acid	CAF	6.0	320
9	Vanillic acid	VAN	6.4	254
10	Syringic acid	Sy	7.9	280
11	*p*-Coumaric acid	*p*-COU	9.5	320
12	Ferulic acid	FER	11.8	320
13	Sinapic acid	SIN	13.9	320
14	Rutin	RUT	20.2	254
15	Oleuropein	OLE	23.9	254
16	Pinoresinol	PIN	26.2	280
17	Quercetin	QUE	27.7	370
18	Luteolin	LUT	30.1	370
19	Kaempferol	KAE	30.8	370
20	Apigenin	API	32.3	320

[a] Slit width wavelength ±4 nm.

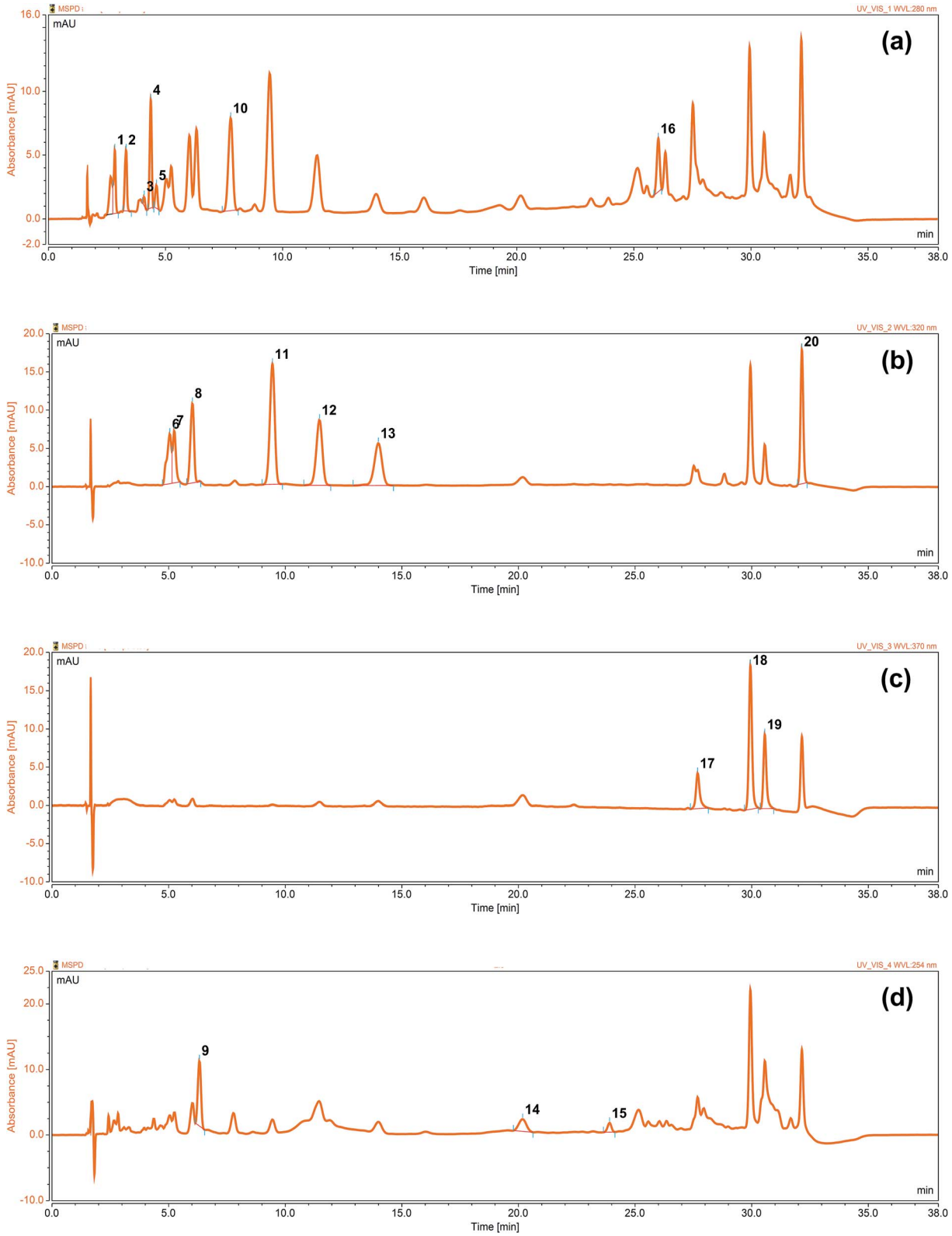

Fig. 1 Typical chromatograms for each detection wavelength for various olive oil samples spiked with 2.5 mg L^{-1} of 20 phenolic compounds. (a) 280 nm, (b) 320 nm; (c) 370 nm and (d) 254 nm. Peak identification numbers: (1) gallic acid; (2) 3-hydroxytyrosol; (3) catechin; (4) tyrosol; (5) 4-hydroxyphenylacetic acid; (6) 2,5-dihydroxybenzoic acid; (7) chlorogenic acid; (8) caffeic acid; (9) vanillic acid; (10) syringic acid; (11) *p*-coumaric acid; (12) ferulic acid; (13) sinapic acid; (14) rutin; (15) oleuropein; (16) pinoresinol; (17) quercetin; (18) luteolin; (19) kaempferol; (20) apigenin.

dissolving an appropriate amount of each compound in HPLC-grade methanol (Merck, Darmstadt, Germany). All the stock solutions were placed under darkness and stored at 4 °C in amber-colored glass bottles.

Formic acid (puriss) was obtained from J.T. Baker (Xalostoc, Mexico) and *n*-hexane was obtained from Merck (Darmstadt, Germany). Florisil (60–100 mesh), Primary-secondary amine (PSA) (50 mesh) and C_{18} (50 mesh) were purchased from United Chemical Technologies UCT, inc. (Bristol, USA). Ultrapure water (18.3 MΩ cm^{-1}) was obtained from Barnstead EASY pure RF water system (Iowa, USA).

2.2 Instrumentation

Chromatographic determination was carried out using a Dionex UltiMate 3000 HPLC system (California, USA) equipped with a LPG-3400M quaternary pump and a MWD detector. The HPLC column used was a reverse-phase chromatography Zorbax Sb-aq (150 mm × 4.6 mm id × 5 μm) from Agilent Technologies (Santa Clara, CA, USA). The column temperature was 40 °C. The Chromeleon 7.1 software was used to control all of the acquisition parameters of the HPLC-MWD system and also to process the obtained data. The mobile phases, A and B, were high-purity water with 0.1% (v/v) formic acid and methanol, respectively. The full gradient program is shown in Table 1. The flow rate was set constant at 1 mL min^{-1} during the entire process, and the injection volume was 10 μL. Prior to use, the mobile phases were filtered through a 0.45 μm membrane filter and degassed. The identification and quantification of the target phenolic compounds in the olive oil samples studied was based on the comparison of the retention times (t_R) and maximum absorbance value of detected peaks in samples of interest with those obtained by the injection of pure standards. The complete information of the t_R and the detection wavelength used for quantification of each analyte are summarized in Table 1. Fig. 1 also shows the chromatogram of various olive oil samples spiked with standard mixture in the optimized conditions. It can be observed that a satisfactory separation of the 20 phenolic compounds was obtained.

2.3 Samples and sample preparation

Olive oil samples studied in this work were provided from local factories in Maipú, Mendoza, Argentina, including different monovarietals (Arauco, Nevadillo, Frantoio, Picual, Manzanilla and Arbequina) and a blend. All the samples were placed in their original containers at ambient temperature, and they were analyzed within the first month after opening. Sample preparation conditions were optimized with aliquots of various olive oil samples ($n = 6$) in the same proportion of different monovarietals and brands (Arauco, Nevadillo, Arbequina and Frantoio) spiked with target analytes at various concentrations. The spiking procedure was carefully carried out in the following manner: an aliquot of methanolic standards solution was dried under nitrogen stream, and then an appropriate portion of sample was added. Similar to this, for example, for a sample with a spiked of 5 mg kg^{-1}, 500 μL of each standard of 1000 mg L^{-1} were pipetted, and after drying, a portion of 100 g of sample was added. Finally, they were shaken for 3 min in a vortex. This procedure was repeated every 3 h to achieve a total of four separate mixing cycles to ensure adequate homogenization. Spiked samples were maintained at room temperature (25 °C) under darkness and used for a maximum of one week.

2.4 Matrix solid-phase dispersion

A portion of 0.5 g of the homogenized sample was placed into a glass mortar and gently blended with 1.0 g of the dispersing agent (Florisil) for 3 min using a pestle to obtain a homogeneous mixture suitable for column packing. This mixture was quantitatively transferred to an empty 5 mL polypropylene syringe with a frit on the bottom. The packing material was covered with another frit and compressed using the syringe plunger and was connected to a vacuum system for solid-phase extraction. A volume of 2.0 mL *n*-hexane was used in order to wash the lipids. After this, complete solvent elimination was insured by positive pressure at the end for 10 min. Finally, 1 mL methanol–water (80 : 20 acidified with formic acid 0.5% (v/v)) was used for the elution of analytes. The eluate was collected in an autosampler amber glass flask and injected into the HPLC-MWD chromatographic system.

2.5 Matrix-matched calibration

The matrix-matched calibration was carried out with different added aliquots of methanolic standard solution in a 50 mL polypropylene tube and was dried under nitrogen stream. After this, an appropriated portion of a pool of sample was added and shaken for 3 min. This shaking procedure was repeated every 3 h to achieve a total of 4 separate mixing cycles to ensure adequate homogenization. After spiking and stabilization, the matrix solid-phase dispersion was performed. Calibration curves comprised eight concentration points for triplicate, within the range from 0 to 25 mg kg^{-1}.

3. Results and discussion

Simplicity is one of the most attractive features of MSPD. Typically, the MSPD method involves blending a solid or semi-solid sample with a rigid and absorbing solid support material, transferring and packing the achieved material into a column, and finally eluting the target analytes. The performance of MSPD is mainly affected by the column packing and elution procedure; thus, it is important to select an appropriate sorbent enabling the homogenization and disruption of samples, simultaneously acting as a separation material. Subsequently, several factors, such as a dispersing sorbent, sorbent-to-sample ratio, cleanup solvent, elution solvents and its volume, influencing the MSPD extraction efficiencies and recoveries of the analytes have been studied.

Prior to MSPD optimization, the chromatographic conditions were studied. According to previous work, methanol resulted in good results for the elution of phenolic compounds.[25] Therefore, different elution gradients with water–methanol mixtures were evaluated to achieve the separation of studied analytes. When high percentages of methanol were

used poor resolution was obtained. Similarly, when the gradient was applied slowly to achieve a methanol percentage of 60%–80%, some peaks appeared to be overlapped. This was principally observed for analytes with t_R longer than 18 min. With the proposed gradient satisfactory resolution of phenolic compounds was obtained.

3.1 Optimization of dispersing sorbent

The effects of extraction and purification for the target analytes from complex matrices are related to the properties of the dispersing sorbents used in MSPD procedures.[16,23] For this reason, sorbents with different chemical properties and different combinations were studied. Similar to this, Florisil and combinations of Florisil with C_{18} and PSA were tested. The experiments were performed maintaining a constant amount of sorbent (1.0 g) and a spiked various sample of 2.5 mg kg^{-1} (0.5 g). As can be seen in Fig. 2 that the best results for the majority of analytes were observed when only Florisil was used. The reason for this could be attributed to the fact that Florisil is a magnesium-based silicate gel, like silica, which is extremely polar and ideal for the isolation of polar compounds (such as most of analytes studied) from non-polar matrices including olive oil, whereas C_{18} is non-polar and PSA has a strong affinity for fatty acids. For this reason, using Florisil plus C_{18} and PSA, lower relative responses were obtained than with Florisil for the great majority of the analytes.

The impact of different ratios of Florisil and olive oil were investigated with the objective of achieving the highest recoveries with the minimum sample and sorbent consumption, as well as for obtaining the required sensitivity for phenolic compounds in olive oils. Various ratios of Florisil to olive oil, ranging from 1 : 1 to 3 : 4 (w/w), were investigated. The optimal ratio of Florisil and olive oil was found to be 2 : 1 (w/w). For some ratios, the sorbent amount was insufficient to properly disrupt and disperse the samples. For example, when the ratios were more than 1 : 2 (w/w), a viscous semi-solid mixture was obtained. In these cases, it was physically impossible to pack the achieved mix into a column and carry out the determination of analytes. On the other hand, the increase in the sorbent quantity did not improve the results. Then, a ratio of 2 : 1

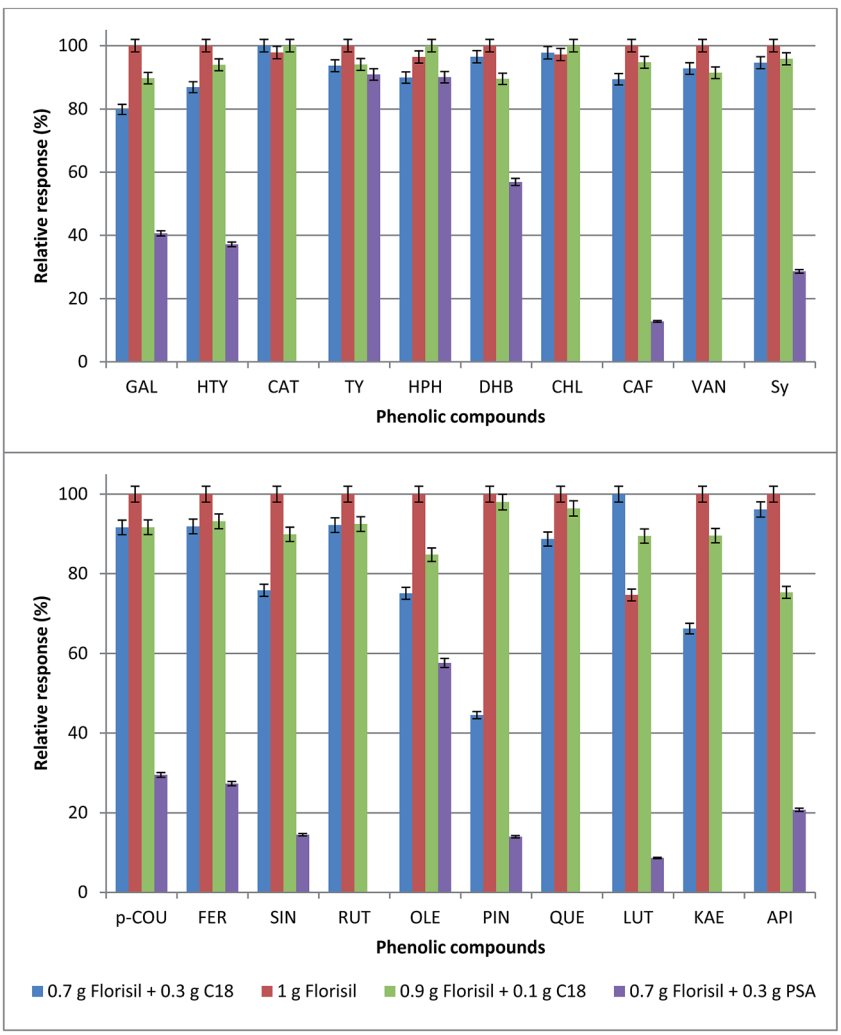

Fig. 2 Evaluation of sorbent on the peak area of 15 phenolic compounds. $n = 3$ replicates.

(1.0 g Florisil and 0.5 g olive oil) was selected to perform further assays.

3.2 Studies on eluting solvents

Lipids may be the main interference in the analysis of minor components in oil samples. Therefore, a rinsing step using *n*-hexane previous to the elution of analytes was performed to eliminate the principal interferences.[26] Without this rinsing step, the obtained extracts were turbid, such as an emulsion because of the presence of lipids. As a result, 2.0 mL of *n*-hexane were used previous to the analyte elution. It is important to mention that the cartridge should be perfectly dried (applied positive pressure at the end of cartridge for 10 min) previous to analyte elution. If *n*-hexane is not totally eliminated, an emulsified eluate will be obtained.

According to previous works,[21,27] methanol and acidified solutions of methanol–water were studied as elution solvents. The experiments were performed using a spiked pool sample of 2.5 mg kg^{-1}. The same volumes of elution agents (1.5 mL) were used in all the experiments. Taking into account the polarity of most of the analytes, the extraction of phenolic compounds was better in water-containing mixtures than in pure organic solvents (see Fig. 3a). The extraction yields increased when the mixture of methanol–water was acidified (80 : 20, v/v with 0.5% v/v of formic acid). This fact could be related to the pK_a of analytes (ranging from 4 to 9). Using this mixture, transparent and colorless extracts were achieved. In a further step, the optimal volume of elution solvent was evaluated. In this case, to obtain comparable results, the volume of eluate was measured. The elution volume was established by collecting consecutive 1 mL fractions of solvent mixture from the cartridge. As shown in Fig. 3b, the highest relative responses for the elution of phenolic compounds from the cartridge were achieved in the first 1 mL fraction. Thus, the first 1 mL fraction of acidified methanol–water solution (80 : 20, v/v with 0.5% v/v of formic acid) was selected as the optimum elution condition for further studies.

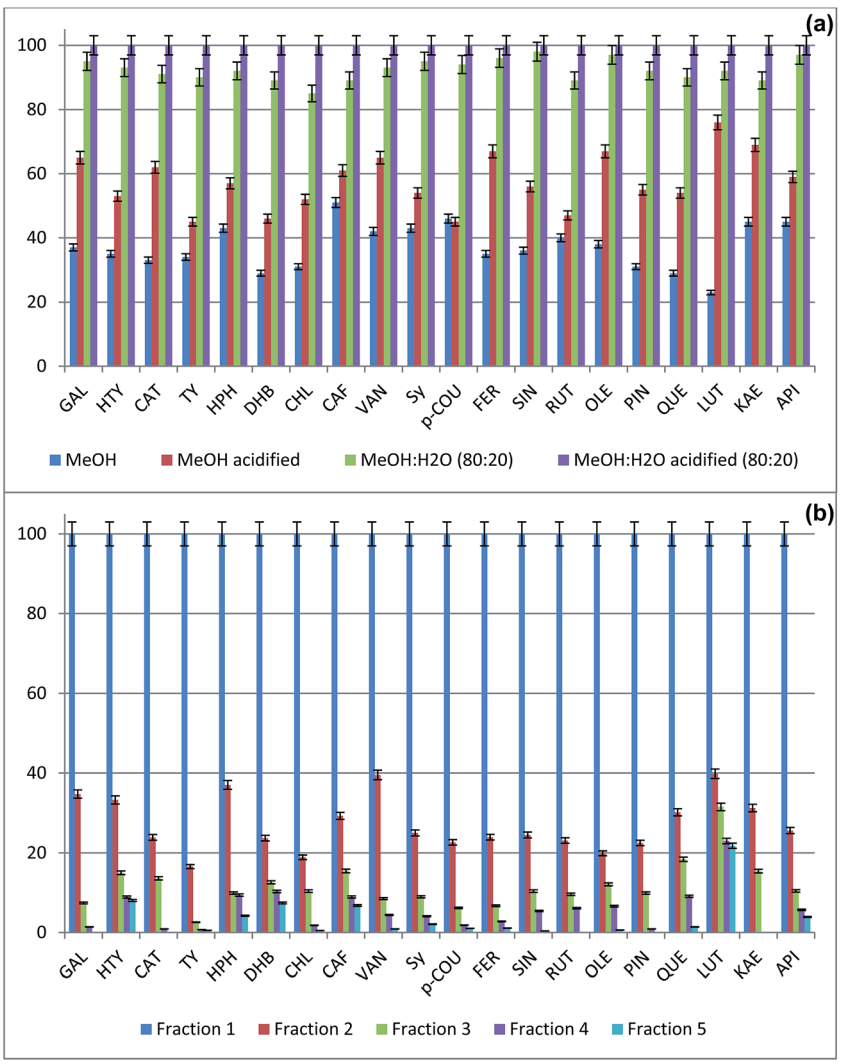

Fig. 3 (a) Effect of elution solvent type on the recovery of phenolic compounds. (b) Evaluation of the elution of analytes studied in consecutive fractions of 1 mL. $n = 3$ replicates.

Table 2 Analytical figures of merit of the optimized methodology

Analyte	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Recovery[a] (%)		Precision[c] (RSD, %)
			1 mg kg^{-1}	5 mg kg^{-1}	
GAL	0.21	0.71	90.2	94.3	3.1
HTY	0.06	0.20	89.9	95.0	4.6
CAT	0.38	1.25	78.3[b]	88.5	9.2
TY	0.02	0.08	93.8	94.6	3.1
HPH	0.27	0.91	79.2	84.5	10.3
DHB	0.75	2.50	83.5[b]	89.7	12.4
CHL	0.33	1.11	76.9[b]	82.3	9.3
CAF	0.13	0.42	77.1	84.6	11.5
VAN	0.25	0.83	94.3	94.9	9.6
Sy	0.16	0.53	92.1	94.8	11.8
p-COU	0.03	0.09	89.6	91.7	2.9
FER	0.03	0.09	84.3	91.4	9.8
SIN	0.13	0.44	80.2	90.5	3.7
RUT	0.17	0.46	90.1	94.1	4.9
OLE	0.27	0.91	89.9	94.3	2.1
PIN	0.25	0.83	93.2	94.1	3.7
QUE	0.17	0.57	74.8	88.2	6.9
LUT	0.19	0.63	89.6	91.9	7.8
KAE	0.38	1.25	77.0[b]	89.1	14.8
API	0.07	0.22	93.5	94.9	9.9

[a] Recovery (%) = 100 × [(found − initial)/added]. Three replicates. [b] Recovery calculated for a spiked level of 2.5 mg kg^{-1}. [c] Calculated on six replicates.

3.3 Performance of the analytical procedure

The analytical figures of merit of the optimized methodology are summarized in Table 2. For estimating the fit of the calibration curves with a linear model, a lack-of-fit test was applied. In this way, the variance of pure error and lack of fit were comparable, indicating a good adjustment with the linear model. In order to evaluate the effect of interferences on the analytical signals of phenolic compounds, the slopes of the calibration graph obtained with matrix-matched standards were compared with those obtained with solvent-based standards, calculating the matrix-to-solvent slope ratios. Each calibration curve comprised eight concentration points for triplicate within the range from 0 to 25 mg kg^{-1}. Depending on the increases or decreases in the values of the slope for each analyte, various matrix effects could be observed: if the value is in the range of 0.85–1.1, the matrix effect could be ignored; if the value is lower than 0.85, it could exhibit a matrix suppression effect; if the value is higher than 1.1, it could show matrix enhancement.[28] It is apparent from Table 3 that the 50% of the investigated analytes do not have a matrix effect, whereas for remaining 50% of compounds, there is a considerable matrix effect. Considering that the difference between both calibration curves was statistically significant, matrix-matched calibration was employed to achieve accurate quantification of the target analytes. For matrix-matched calibration curves, the linear range was between near to LOD concentration until 15 mg kg^{-1}.

Precision and accuracy were assessed using various olive oil spiked at two different concentration levels: 1 and 5 mg kg^{-1}. In all the cases, spiked and non-spiked aliquots were processed in triplicate, and the concentrations of phenolic compounds in the corresponding extracts were determined by matrix-matched standards calibration. The recoveries (R, %) of the overall procedure, considered as an estimation of the accuracy, for 0.5 g samples ranged between 74.8% and 95.0% for either additional levels. In both the cases, the associated standard deviations

Table 3 Results of solvent and matrix calibration for matrix-effect studies

Analyte	Solvent calibration		Matrix calibration		Slope matrix/slope solvent
	Slope	R^2	Slope	R^2	
GAL	0.427	0.999	0.092	0.997	0.22
HTY	0.125	0.998	0.129	0.999	1.0
CAT	0.075	0.999	0.033	0.999	0.44
TY	0.126	0.999	0.109	0.992	0.87
HPH	0.163	0.998	0.113	0.981	0.69
DHB	0.209	0.995	0.316	0.995	1.5
CHL	0.864	0.999	0.327	0.994	0.38
CAF	0.763	0.999	0.661	0.994	0.87
VAN	0.595	0.999	0.509	0.994	0.86
Sy	0.567	0.998	0.600	0.999	1.1
p-COU	1.403	0.990	1.078	0.992	0.77
FER	0.916	0.998	0.808	0.995	0.88
SIN	0.800	0.998	0.609	0.996	0.76
RUT	0.276	0.999	0.250	0.993	0.91
OLE	0.098	0.997	0.110	0.994	1.1
PIN	0.101	0.999	0.200	0.999	2.0
QUE	0.532	0.997	0.407	0.996	0.77
LUT	1.028	0.995	0.983	0.990	0.96
KAE	0.601	0.999	0.516	0.997	0.86
API	0.026	0.999	0.680	0.997	26

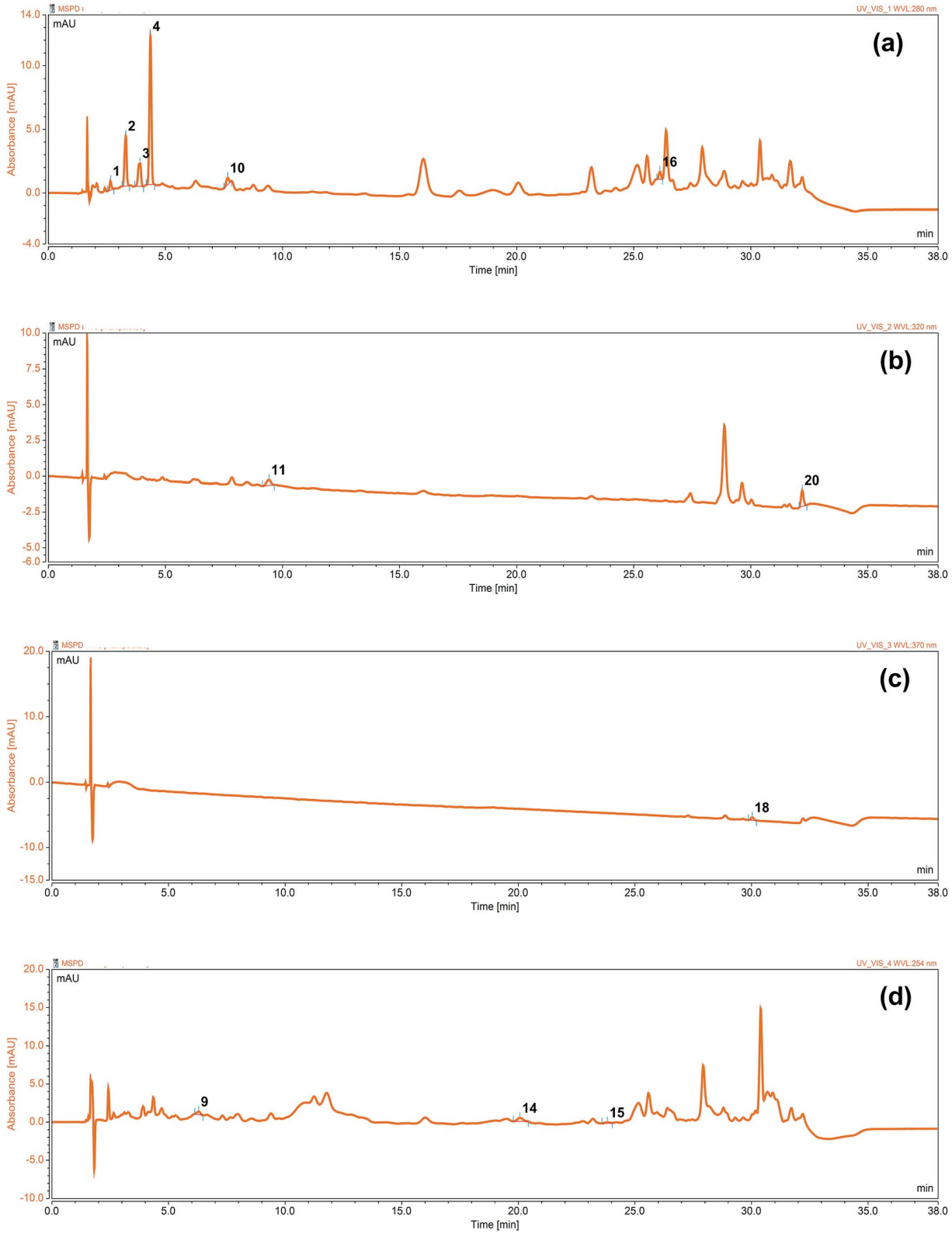

Fig. 4 Chromatograms of each detection wavelength for a blend sample: (a) 280 nm, peak identification: (1) gallic acid; (2) 3-hydroxytyrosol; (3) catechin; (4) tyrosol; (10) syringic acid and (16) pinoresinol. (b) 320 nm, peak identification: (11) *p*-coumaric acid and (20) apigenin. (c) 370 nm, peak identification: (18) luteolin. (d) 254 nm, peak identification: (9) vanillic acid, (14) rutin and (15) oleuropein.

varied between 1.5% and 9.3%. The RSD resulting from the analysis of six replicates of 0.5 g of a sample pool containing 2.5 mg kg^{-1} phenolic compounds were ranged between 2.1% (for OLE) and 14.8% (for KAE). Table 2 illustrates an overview of the precision and accuracy data.

The LODs of the proposed methodology, defined for an *S*/*N* of 3 were estimated from *S*/*N* values of the target species in a spiked various sample of 0.1 mg kg^{-1} and were between 0.02 for TY and 0.75 mg kg^{-1} for DHB. The LOQs of the method, defined for an *S*/*N* of 10, were comprised between 0.08 and 2.50 mg kg^{-1} (Table 2). The achieved LODs showed that the proposed MSPD-HPLC-MWD method shows a suitable sensitivity according to the phenolic compound levels commonly found in olive oils.

Finally, the results obtained with the developed methodology were compared with the reference method of the International Olive Council (IOC) "Determination of biophenols in olive oils by HPLC".[29] Various sample were analyzed by the two methodologies with the aim of verifying the efficacy of the new method. The obtained results, in terms of total biophenol content, expressed as mg kg^{-1} of TY, were statistically comparable (15.07 ± 0.36 and 14.68 ± 0.31 for MSPD and IOC methods, respectively). In this way, the new methodologies show important advantages in terms of time consumption. Whereas the IOC method nearly requires 42 min for the extraction of each sample, the MSPD-HPLC-MWD needs only 13 min for the same procedure. In terms of solvent consumption for the extraction/cleanup procedure, the IOC methodology requires about twice the amount of solvent, compared with our methodology. In addition, when considering the separation step, the run time for each sample is markedly superior for the IOC method (82 min, compared with 38 min) with the same flow. Thus, each analysis for the IOC method consumes more solvent and, as a result, a high volume of waste is produced. On the other hand, the new methodology needs a greater number of standards. The IOC method quantifies the biophenols content, expressed as mg kg^{-1} of TY, and identifies each analyte by using the relative retention time (RRT) according to Sy (internal standard). Taking this into account, the proposed methodology allows the quantification of individual phenolic compounds giving more detailed information about each sample.

3.4 Samples

The optimized method was used to investigate the levels of phenolic compounds in olive oil samples from different varieties cultivated in Argentina. Fig. 4 shows the chromatograms obtained for a blend sample. It can be observed that good peak shape and resolution were achieved for all compounds. Table 4 summarizes the concentrations of phenolic compounds measured for triplicate in each olive oil sample. LUT, TY, API and *p*-COU were found in all the samples. In addition, HTY and TY were found in higher concentrations compared with other compounds, such behavior previously reported by other authors.[30] TY was quantified in all the samples in a range from 0.76 to 15.32 mg kg^{-1} for Frantoio and Arauco 4, respectively, whereas the levels for LUT were under quantification limit in eight samples and can be only quantified in a blend sample at levels near that of LOQ. The quantification of API was possible in three samples (Arauco 1, 2 and 3), whereas in the remaining samples it remains under the LOQ. *p*-COU was only quantified in the Arauco 3 sample. Sy was found in eight of nine samples.

Table 4 Determination of phenolic compounds in virgin olive oil samples

	Sample (mg kg^{-1})								
Analyte	Blend	Arauco 1	Arauco 2	Arauco 3	Arauco 4	Picual	Arbequina	Manzanilla	Frantoio
GAL	0.76 ± 0.04	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.q.[b]	n.q.[b]	n.q.[b]	n.d.[a]
HTY	3.22 ± 0.14	0.47 ± 0.03	0.54 ± 0.04	1.06 ± 0.08	3.61 ± 0.27	0.59 ± 0.04	n.d.[a]	2.47 ± 0.18	2.10 ± 0.16
CAT	7.54 ± 0.45	n.d.[a]	n.d.[a]	n.d.[a]	3.84 ± 0.23	n.d.[a]	0.82 ± 0.05	n.d.[a]	n.d.[a]
TY	10.92 ± 0.23	5.93 ± 0.01	6.39 ± 0.04	11.11 ± 0.12	15.32 ± 0.13	4.26 ± 0.01	0.76 ± 0.01	4.34 ± 0.02	2.89 ± 0.02
HPH	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
DHB	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.q.[b]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
CHL	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
CAF	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
VAN	n.q.[b]	0.84 ± 0.03	n.d.[a]	n.d.[a]	n.d.[a]	0.94 ± 0.02	0.84 ± 0.04	n.d.[a]	n.d.[a]
Sy	n.q.[b]	n.q.[b]	n.q.[b]	n.d.[a]	n.q.[b]	0.61 ± 0.02	n.q.[b]	0.83 ± 0.02	n.q.[b]
p-COU	n.q.[b]	n.q.[b]	n.q.[b]	0.11 ± 0.01	n.q.[b]	n.q.[b]	n.q.[b]	n.q.[b]	n.q.[b]
FER	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.q.[b]	n.d.[a]	n.d.[a]
SIN	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.q.[b]	n.d.[a]	n.d.[a]
RUT	0.46 ± 0.02	n.d.[a]	0.46 ± 0.02	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	0.86 ± 0.04	0.89 ± 0.04
OLE	0.93 ± 0.02	n.d.[a]	n.d.[a]	n.d.[a]	1.68 ± 0.04	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
PIN	0.84 ± 0.02	0.84 ± 0.02	0.84 ± 0.02	n.d.[a]	1.23 ± 0.06	1.22 ± 0.06	0.85 ± 0.03	0.86 ± 0.03	0.84 ± 0.02
QUE	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
LUT	0.64 ± 0.03	n.q.[b]	n.q.[b]	n.q.[b]	n.q.[b]	n.q.[b]	n.q.[b]	n.q.[b]	n.q.[b]
KAE	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.q.[b]	n.q.[b]
API	n.q.[b]	0.30 ± 0.04	0.23 ± 0.02	0.24 ± 0.02	n.q.[b]	n.q.[b]	n.q.[b]	n.q.[b]	n.q.[b]
T.C.[c]	25.23	8.37	8.47	12.52	26.46	8.25	3.57	10.19	6.72

[a] Not detected. [b] Under quantification limit. [c] Total concentration expressed in mg kg^{-1}.

However, in most of the samples, the concentrations were between LOD and LOQ, whereas in Picual and Manzanilla, it was possible to perform the quantification. On the other hand, the sum of phenolic compound concentration was highest in the Arauco 4 sample (26.46 mg kg^{-1}). These results are in concordance with those previously reported by Ceci, L. *et al.* informed total phenolic compounds, finding the highest values for this varietal. However, the levels reported by Ceci, L. *et al.* were higher than those found in the present work.[31] HPH, CHL, CAF and QUE were not detected in any sample, whereas DHB, FER, SIN and KAE were found in some samples at concentration levels between LOD and LOQ, (DHB in Arauco 4; FER and SIN in Arbequina; and KAE in Manzanilla and Frantoio). Finally, as previously mentioned, the total content of phenolic compounds is low, compared with olive oils of other countries. This has been explained as a poor adaptation of some cultivars to local agroclimatic conditions and a non-optimized control in processing parameters, such as temperature and time, in the beating process.[30,31]

4. Conclusions

In the present work, a MSPD-HPLC-MWD methodology for the determination of phenolic compounds in the complex matrix of olive oil was developed. The methodology allows for the selective determination of 20 phenolic compounds in olive oil samples with satisfactory sensitivities, recoveries and RSDs, which are compatible with levels present in the samples.

The extraction/cleanup procedure of the described method is simple, requiring little sample preparation and allowing for the increase of sample throughput.

The applicability of the methodology was demonstrated by the analysis of nine olive oil samples. A good performance of the method was observed, allowing the reliable determination of the target compounds in such non-polar samples.

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