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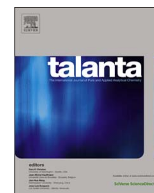

Quantitative method for determination of oleocanthal and oleacein in virgin olive oils by liquid chromatography–tandem mass spectrometry

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ABSTRACT

Oleocanthal and oleacein, two key secoiridoid derivatives present in virgin olive oil (VOO), are gaining clinical and nutritional interest thanks to their proved bioactivity; therefore, the determination of both phenols is a growing demanded application to increase the value of VOO. The main problem of previously reported liquid chromatography-based methods for oleocanthal and oleacein measurement is their interaction with water or other polar solvents such as methanol to promote the formation of hemiacetal or acetal derivatives. This interaction can occur during either sample extraction, basically liquid–liquid extraction, and/or chromatographic separation. The aim of this research was to evaluate the suitability of LC–MS/MS for absolute quantitation of oleocanthal and oleacein in VOO. For this purpose, both liquid–liquid extraction and chromatographic separation were studied as potential promoters of acetals and hemiacetals formation from oleocanthal and/or oleacein. The results showed that the use of methanol–water solutions for phenols extraction was not influential on the formation of these artifacts. Acetals and hemiacetals from oleocanthal and/or oleacein were only detected at very low concentrations when methanol gradients under acidic conditions were used for chromatographic separation. With this premise, a protocol based on extraction with acetonitrile and a reverse chromatographic gradient with methanol was established to quantify in absolute terms oleocanthal and oleacein in VOO samples. The resulting protocol was applied to three VOO samples characterized by high, medium, and low levels of these two phenols.

1. Introduction

Virgin olive oil (VOO) contains multiple minor components, such as sterols, volatile compounds, and phenols, among the most important families. Olive oil phenols comprise acids, phenolic alcohols, such as tyrosol (abbreviated as *p*-HPEA) and hydroxytyrosol (3,4-DHPEA), flavonoids, lignans, and secoiridoids (oleuropein, ligstroside and their derivatives). The bioactive capability of phenols present in VOO is a matter of great interest because of the proved or tentatively described healthy effects attributed to them. Additionally, olive oil phenols are major contributors to the long shelf-life and organoleptical characteristics of VOO [1,2]. Two secoiridoid derivatives should be mentioned in this regard, the dialdehydic forms of decarboxymethyl ligstroside and oleuropein aglycones, also known as oleocanthal (*p*-HPEA-EDA), and oleacein (3,4-DHPEA-EDA), respectively (Supplementary Fig. 1) [3]. These compounds are endowed with antimicrobial, anticancer, and hypoglycemic effects, and are considered key oxidation inhibitors among the main responsible for the antioxidant properties of VOO [4]. It is noteworthy to point out that oleacein has been declared a more potent antioxidant than hydroxytyrosol [4]; furthermore, the interest in these derivatives has been enhanced because of their reported anti-inflammatory properties. Thus, oleocanthal has shown intense anti-inflammatory effects comparable to ibuprofen thanks to its capability to inhibit cyclooxygenases COX-1 and COX-2 but not 15-lipooxygenase [5]. Indeed, some authors have pointed out that oleocanthal is one of the main components responsible for the therapeutic properties of VOO [6]. Recently, oleocanthal has also been proposed as a promising agent to induce selectively cancer cell death via lysosomal membrane permeabilization [7]. Concerning sensory properties, oleocanthal is responsible for the burning pungent sensation of VOO [8].

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Due to the relevance of these two secoiridoid derivatives the quantitative analysis of them can provide an added value to VOOs and, therefore, an attractive aim of olive breeding programs. However, their quantitation is a pending goal of the characterization of olive oils due to the lack of both knowledge about them and commercial standards. Several methods have been described for analysis of oleocanthal and oleacein in VOO, mainly based on liquid chromatography (LC) separation followed by UV–Vis or mass spectrometry (MS) detection [5,9,10], and by quantitative NMR [11]. Methanol–water mixtures are commonly used for the extraction of phenols from VOO due to their mid-polar character. Nevertheless, some authors have proposed the use of acetonitrile (ACN) since it provides better extraction efficiency than methanol (MeOH) for isolation of secoiridoids and derivatives such as oleocanthal [12]. Recently, researchers have identified a limitation in the determination of oleocanthal and oleacein by LC-based methods explained by the reaction of these dialdehydic compounds with water or MeOH, both used in the extraction step and in the mobile phases for chromatographic separation. Hence, Karkoula et al. [11] studied the reaction of oleocanthal and oleacein with water, MeOH, ACN, chloroform ($CHCl_3$), dimethyl sulfoxide (DMSO), and their mixtures by NMR using deuterated solvents and monitoring the formation of acetals and hemiacetals (Supplementary Fig. 2). Oleocanthal and oleacein provided NMR spectra that corresponded each to a single molecule only in the case of deuterated chloroform, ACN or DMSO; instead, hemiacetal and acetal derivatives were generated in water or MeOH–water mixtures. No studies dealing with the stability of oleocanthal and oleacein and the formation of hemiacetal and acetal derivatives by LC–MS/MS analysis have so far been reported. This fact could explain the lack of LC-based methods for quantitative analysis of oleocanthal and oleacein in VOO. In the present research, the two main steps (viz., liquid–liquid extraction and chromatographic analysis) that could potentially interfere in the determination of oleocanthal and oleacein by LC–MS/MS have been studied. After this study, a method for absolute quantitation of oleocanthal and oleacein by LC–MS/MS has been proposed.

2. Materials and methods

2.1. Monovarietal virgin olive oil samples

Three monovarietal olive oils from Arbequina (Córdoba, Spain), Picual (Jaén, Spain), and Lianolia Kerkiras (Corfu, Greece) cultivars obtained in the 2014/2015 season were used in this research. Olive fruits were collected in 2014 at intermediate ripening (when the fruit color is changing from yellowish with reddish spots to reddish) from cultivars located in different places. The selection of these varieties was supported on their content in oleocanthal and oleacein described in previous papers [13,14].

2.2. Reagents

The solvents used for analysis of oleocanthal and oleacein in VOOs were LC–MS grade MeOH, ACN, and *n*-hexane, which were from Scharlab (Barcelona, Spain). LC–MS-grade formic acid, also from Scharlab, was used as ionization agent in the chromatographic mobile phases. Deionized water (18 MΩ cm) from a Millipore Milli-Q water purification system (Bedford, MA, USA) was used to prepare both the aqueous mobile phase and the hydroalcoholic mixture used as extractant.

Oleocanthal and oleacein (purity > 98%) were isolated from a VOO extract prepared using the protocol for extraction, purification and characterization described by Karkoula et al. [11,14]. Standard solutions of both compounds (1 mg/mL) were prepared in pure acetonitrile to preserve their stability.

2.3. Apparatus and instruments

An MS2 minishaker from Ika Works (Wilmington, NC, USA) was used to enhance the transfer of phenols from VOOs to the tested extractants prior to quantitation of oleocanthal and oleacein. Phenolic extracts were analyzed by an 1200 Series LC system (Agilent Technologies, Waldbronn, Germany) coupled to an Agilent 6460 triple quadrupole (LC–QqQ-MS) detector furnished with an electrospray ionization (ESI) source. A confirmatory analysis in accurate mode of the two secoiridoids and the corresponding hemiacetals and acetals was conducted by an 1200 Series LC system coupled to an Agilent 6540 quadrupole-time-of-flight (LC–QTOF-MS) hybrid mass spectrometer with a Dual ESI source for simultaneous spraying of the LC eluent and a reference mass solution enabling continuous calibration of detected *m/z* ratios.

2.4. Extraction of oleocanthal and oleacein from VOOs extracts

Two extraction procedures (both based on shaking VOO solutions in hexane with either ACN or an MeOH–water mixture) were applied to isolate both phenols from VOO samples. Thus, 1 g of VOO was diluted with 1 mL of hexane and shaken for 1 min with either 1 mL of a 60:40 (v/v) MeOH–water mixture or 1 mL of ACN. The hydroalcoholic or ACN phase was separated by centrifugation and the extraction process was repeated to attain quantitative extraction as described by Hrncirik and Fritsche [15]. The resulting phenolic extracts were 1:2 or 1:50 diluted, depending on the content of secoiridoid derivatives, and analyzed by LC–QTOF and LC–QqQ MS/MS.

2.5. LC–QTOF MS/MS confirmatory analysis of oleocanthal, oleacein, and acetal forms in extracts from VOO

Identification of the two olive phenols and the hemiacetal and acetal artifacts was conducted by LC–QTOF MS/MS confirmatory analysis in accurate mode. Analyses were performed by reversed-phase liquid chromatography followed by electrospray ionization (ESI) in negative mode and tandem mass spectrometry (MS/MS) detection. Five µL of extract was injected in triplicate into the LC system for chromatographic separation of the target compounds using a C18 Pursuit XRs Ultra (50×2.0 mm i.d., 2.8 µm particle size) from Varian (Walnut Creek, CA, USA). The column compartment was kept at 30 °C. Mobile phase A was 0.1% formic acid in water, while phase B was 0.1% formic acid in MeOH. The gradient program, at 0.4 mL/min constant flow rate, was as follows: initially 50% phase A and 50% phase B kept for 0.5 min; from 0.5 to 2 min was from 50% to 20%; from 2 to 4 min, mobile phase A was from 20% to 0% A. This last composition was kept for 1 min. After each analysis, the column was equilibrated for 5 min to the initial conditions and pressure equilibration. The total running time of the analysis was 10 min.

The electrospray ionization source was operated in the negative ionization mode, and the flow rate and temperature of the drying gas (N_2) were 10 L/min and 350 °C, respectively. The nebulizer pressure was 35 psi, and the voltages of the capillary, skimmer, and octopole radiofrequency were 3250, 65, and 90 V, respectively. The focusing voltage set in the first quadrupole was 90 V. The data were acquired in centroid mode in the extended dynamic range (2 GHz). Full scan with subsequent activation of the three most intense precursor ions per scan (only single or double charged ions were allowed) by tandem mass spectrometry (MS/MS) was carried out at 1 spectrum/s in the *m/z* range 50–1700. Three values for collision energy of (15, 20, and 25 eV) were tested by independent runs to increase the MS/MS information for identification of oleocanthal, oleacein, and their acetal and hemiacetal derivatives. An active exclusion window was programmed after one MS/MS spectrum and released after 0.75 min to avoid repetitive fragmentation of the most intense precursor ions and, in this way, increase the detection coverage. Before the experiments, the instru-

ment reported mass detection resolution of 25000 full width at half maximum (FWHM) at m/z 112.9856 and 45,000 FWHM at m/z 966.0007. To assure the desired mass accuracy of recorded ions, continuous internal calibration was performed during analyses with the use of signals at m/z 119.0363 (proton abstracted purine) and at m/z 966.0007 (formate adduct of hexakis(1 H,1 H,3H-tetrafluoropropoxy)phosphazine). Identification of the compounds and their product ions proceeded by generation of candidate formulae with a mass accuracy limit of 5 ppm.

2.6. LC–QqQ MS/MS analysis of oleocanthal and oleacein in extracts from VOOs

Quantitative analysis was carried out by LC–QqQ MS/MS after identification of both phenols in VOO. The analytical column, mobile phases with the substitution of MeOH as phase B, and gradient program were those used for qualitative analysis by LC–QTOF. The volume of injected extract was also 5 µL. The entire eluate was electrosprayed and monitored by MS/MS in Selected Reaction Monitoring (SRM) mode of selective transitions from precursor to product ions for each analyte. The flow rate and temperature of the drying gas (N_2) were 10 L/min and 300 °C, respectively. The nebulizer pressure was 50 psi and the capillary voltage 3000 V. The dwell time was set at 200 ms/spec.

2.7. Quantitation of the target compounds

Absolute quantitative analysis was performed by preparing calibration curves using refined olive oil spiked with oleocanthal and oleacein standards. The absence of quantifiable levels of both phenols in the refined oil was checked by direct analysis with the developed method. Nine concentrations from 0.01 µg/mL to 5 µg/mL were injected in triplicate to obtain the calibration curves. The concentrations of oleacein and oleocanthal in the monovarietal VOOs were determined with these models using three replicates per sample. Concerning the acetals and hemiacetals formed during analysis, they were relatively quantified by using the calibration model of the corresponding phenol.

3. Results and discussion

3.1. Determination of oleocanthal and oleacein in VOO

Quantitative analysis of oleocanthal and oleacein in VOOs by LC-based methods suffers from the limitations described by some authors regarding to formation of hemiacetal or acetal derivatives which can interfere in the analysis of these oleopentanedialdehydes [11,16]. Karkoula et al. [11] reported that 96% of the methyl hemiacetals (Supplementary Fig. 2) was generated in MeOH or 1:1 MeOH–water mixture as solvent, while the oleocanthal and/or oleacein monohydrates were detected only when water was used. According to these results the authors developed a method for direct measurement of oleocanthal and oleacein by ^{1}H NMR without involvement of any potentially reactive solvent.

To confirm the presence of oleocanthal and oleacein in monovarietal VOO samples included in the present study, analysis of the two pure standards by LC–QTOF MS/MS was first programmed using for the separation of the peaks the MeOH chromatographic gradient above mentioned. Oleacein and oleocanthal are characterized by the same dialdehydic structure, the only difference between them being the phenolic moiety, hydroxytyrosol and tyrosol, respectively. Extracted ion chromatograms (EIC) for $[M-H]^-$ ions from standards of oleocanthal with m/z 303.1238 and oleacein with m/z 319.1181 showed two peaks at 1.45 (Fig. 1a) and 1.00 min (Fig. 1b), respectively, which were clearly identified by MS/MS fragmentation. Fragmentation of the precursor ion m/z 303.1238 generated five representative product ions, two of which, detected at m/z 137.0608 and m/z 119.0505, corresponded to tyrosol and its principal fragment when activated by MS/MS. Two other fragments were detected at m/z 139.0767 and m/z 123.0445, which were assigned to the dialdehydic moiety, released after separation of the tyrosol, and its main fragment, respectively, as shows Fig. 1a. The fifth ion at m/z 59.0135 fit the acetoxy fragment associated to the ester bound. Fig. 1b illustrates the fragmentation of oleacein that led to two main ion products at m/z 139.0767 and at m/z 59.0135 corresponding to the dialdehydic moiety and the acetoxy fragment released after separation of hydroxytyrosol by analogy to oleocanthal. Besides, one ion at m/z 123.0448 was clearly identified as the hydroxytyrosol main fragment, which allowed confirming the identity of oleacein. Fig. 2 shows the EICs corresponding to both phenols provided by analysis of a VOO sample after liquid–liquid extraction with 60:40 (v/v) MeOH–water. The analysis of the hydroalcoholic extract from the VOO sample also allowed detecting the presence of acetals and hemiacetals from oleocanthal and oleacein, which were identified by virtue of the same fragmentation patterns described for their precursors. The dimethyl acetal of oleacein was detected at m/z 365.1500, while the analog for oleocanthal was not detected at its m/z value at 349.1651. Concerning hemiacetal derivatives, only the methyl hemiacetals were found in the hydroalcoholic extracts from VOO. The oleocanthal and oleacein methyl hemiacetals were found at m/z 335.1500 and m/z 351.1449, respectively. The MS/MS spectra of acetals and hemiacetals were characterized by the presence of representative fragments of oleocanthal and oleacein at m/z 137.0627 and m/z 123.0448, respectively. Apart from that, fragments at m/z 139.0739 and m/z 59.0135, which are also typical from the structure of these secoiridoid derivatives, were detected. Fig. 2 also shows the MS/MS spectra obtained from the methyl hemiacetals from oleocanthal and oleacein and the dimethyl acetal from oleacein. A mass difference in the acetals/hemiacetals MS/MS spectra was observed by loss of 14 Da, which fits the cleavage of the methyl group with the formation of the hydroxyl group..

After confirming the presence of oleocanthal and oleacein in VOO and verifying the formation of hemiacetals and acetals during LC–QTOF MS/MS analysis of hydroalcoholic extracts, an optimization study was designed to develop an MS/MS method based on SRM by LC–QqQ MS/MS. The selection of the SRM transitions and the corresponding acquisition parameters (e.g. the isolation voltage of the first quadrupole and collision energy) were optimized by using phenolic extracts from monovarietal VOOs. The most sensitive transitions from precursors to product ions were used for quantitation of oleocanthal and oleacein, and the corresponding hemiacetals and acetals; whereas secondary transitions were used for confirmatory analysis. A summary of the SRM method is listed in Table 1 that also includes the calibration models, limits of detection and quantitation (LODs and LOQs, respectively), and precision estimated as within-day variability (expressed as percentage of relative standard deviation).

3.2. Influence of sample preparation on the determination of oleocanthal and oleacein

MeOH–water mixtures (the exact composition depending on the target phenols) are frequently used as extractant for isolation of phenolic compounds from VOO. Thus, hydroxytyrosol and tyrosol, with polar character, are better extracted by mixtures with a high concentration of water, while flavonoids and secoiridoids demand for a high proportion of organic solvent. With these premises, the most used extractant composition for isolation of phenols from VOO is 60:40 (v/v) MeOH–water. On the other hand, LC–MS/MS analyses are mainly carried out with reversed-phase gradients from aqueous to methanolic phase under acidic conditions, usually with formic acid, to enhance the ionization of phenols prior to MS detection. Therefore, two potential steps can be involved in the formation of acetals and hemiacetals: extraction and chromatographic separation.

The first study was aimed at knowing the influence of the phenols

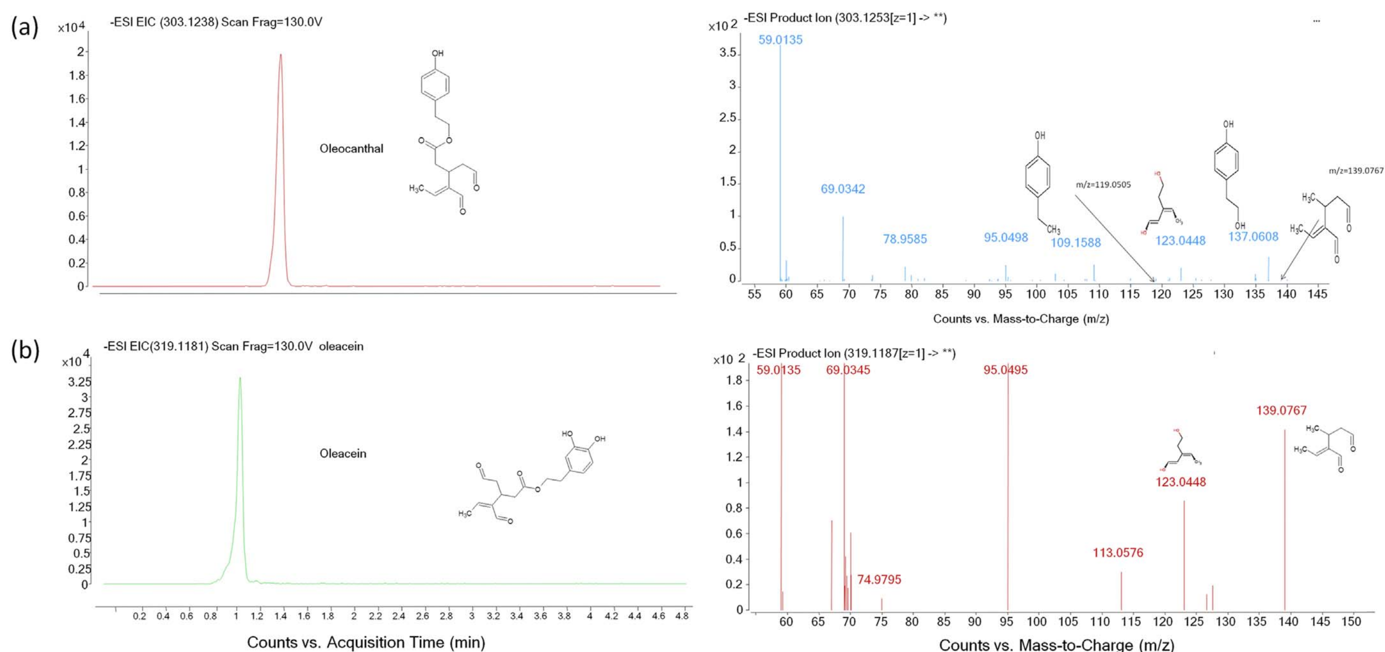

Fig. 1. Extracted ion chromatograms (EICs) and MS/MS spectra of (a) oleocanthal and (b) oleacein standards by LC–QTOF analysis.

Fig. 2. Extracted ion chromatograms (EICs) and MS/MS spectra provided by LC–QTOF analysis of a MeOH–water extract from Picual VOO using the MeOH-based gradient: (a) oleacein and its methyl hemiacetal and dimethyl acetal; (b) oleocanthal and its methyl hemiacetal.

extraction procedure on the formation of hemiacetals and acetals from oleocanthal and oleacein. For this purpose, MeOH–water extracts were analyzed by LC–QqQ MS/MS in SRM mode to evaluate the formation of derivatives by comparison with extracts obtained with ACN, which does not promote the formation of derivatives from oleocanthal and oleacein. A chromatographic gradient based on ACN was used to minimize the formation of acetals and hemiacetals by LC–MS/MS analysis. Fig. 3 shows the SRM chromatograms obtained by analysis of MeOH–water and ACN extracts from Arbequina and Picual VOOs representing the behavior of the three analyzed monovarietal oils. As

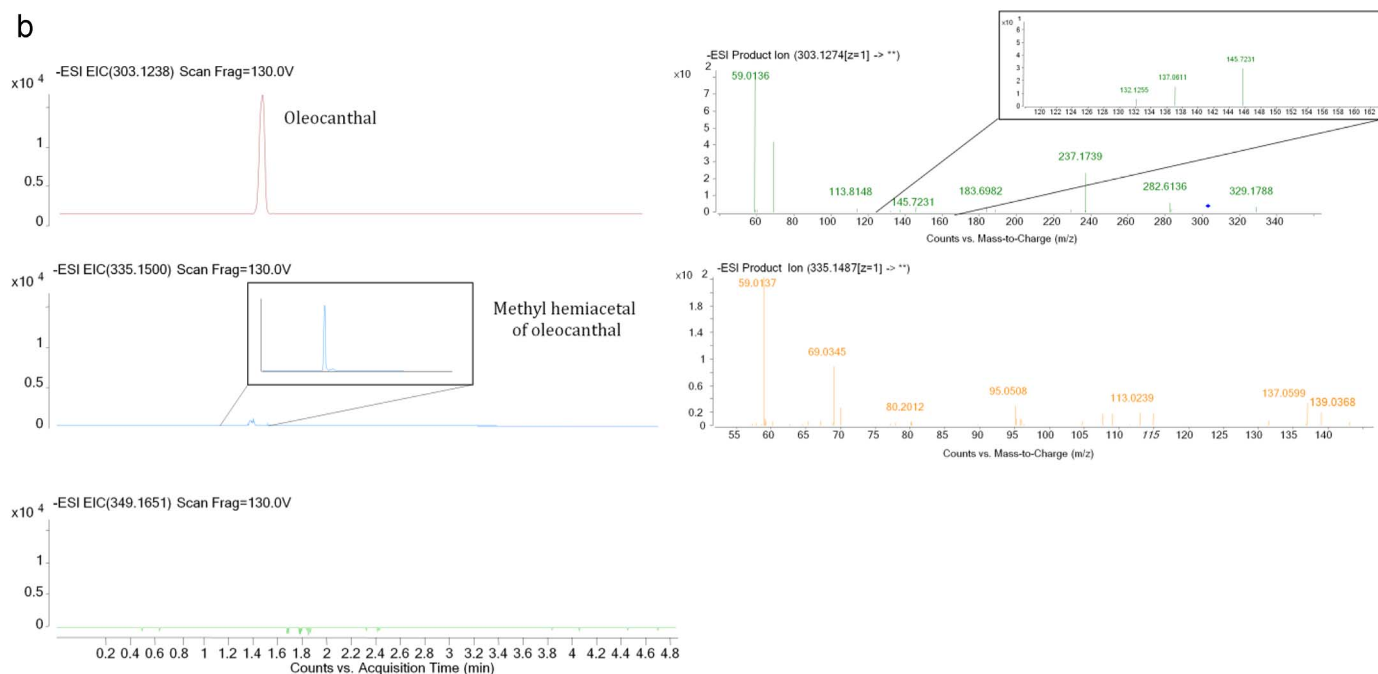

Fig. 2. (*continued*)

can be seen, the formation of acetal and hemiacetal derivatives in the extract was not detected by LC–QqQ MS/MS. The presence of peaks in the extracted ion chromatograms corresponding to the transition 349→137, for monitoring the dimethyl acetal of oleocanthal, is due to the formation of formic acid adducts of oleocanthal. These results allowed deducing that the formation of acetal/hemiacetal artifacts was not influenced by extraction with hydroalcoholic mixtures under these conditions. Additionally, the quantitative responses led to the conclusion that ACN provided similar extraction efficiency as MeOH–water for phenols in VOOs (data not shown).

Methanolic extracts from VOOs were analyzed again after three months storage at −20 °C. These analyses allowed discarding the formation of acetals and hemiacetals during the storage period, as shows Supplementary Fig. 3; that is, the reaction did not progress when the extracts are stored at −20 °C.

3.3. Influence of the chromatographic method on the determination of oleocanthal and oleacein

The influence of the mobile phase on the conversion of oleocanthal and oleacein into hemiacetal and acetal derivatives was also studied. For this purpose, two chromatographic gradients using MeOH and ACN as organic solvents (phase B) were tested for analysis of phenolic extracts obtained with MeOH–water or ACN as extractants. Table 2 shows the relative concentrations, expressed as percentage, as obtained for each compound under the tested experimental conditions. As can be seen, acetals and hemiacetals of oleocanthal and oleacein were only detected at very low concentrations with methanol-based gradients, as also reveals Fig. 4 for ACN extracts. This could be explained by the acidic pH used in the chromatographic separation according to De Nino et al. [17], who found enhanced formation of acetal derivatives in acid media. The formation of methyl hemiacetals was slightly favored over that of oleocanthal and oleacein monohydrates. In fact, in this work the formation of the monohydrate forms was not observed, which is in agreement with the results obtained by Karkoula et al. [11]. In relative terms, the free form of oleacein constituted 93.9 ± 0.2% of its total concentration in the extracts from VOO samples using MeOH-based chromatographic gradients and ACN extraction, while its methyl hemiacetal represented 5.1 ± 0.3% and the dimethyl acetal derivative was only detected in VOO at 1.0 ± 0.2%. On the other hand, the relative concentration of oleocanthal was estimated around 90.2 ± 1.5% in the ACN extracts from VOOs, while the methyl hemiacetal represented 9.8

(A) Optimization of the LC–QqQ MS/MS step for qualitative and quantitative determination of oleacein and oleocanthal.

Compound	Precursor ion	Q1 voltage (V)	Collision energy (eV)	Quantitative transition (*m/z*)	Product ion confirmation (*m/z*)
Oleacein	319	110	18	319→123	59, 137, 139
Methyl hemiacetal of oleacein	351	110	18	351→123	139, 59
Dimethyl acetal of oleacein	365	110	18	365→123	139, 59
Oleocanthal	303	110	18	303→137	119,139,59
Methyl hemiacetal of oleocanthal	335	110	18	335→137	139, 59
Dimethyl acetal of oleocanthal[*]	349	110	18	349→137	139,59

(B) Analytical features of the method for quantitative determination of oleacein and oleocanthal in olive oils by LC–QqQ MS/MS.

Compound	Calibration model	Coefficient of regression (R^2)	LOD (µg/mL)	LOQ (µg/mL)	Within day variability (RSD)
Oleacein	$y = 5749.8x + 306.7$	0.992	0.002	0.005	11%
Oleocanthal	$y = 2778.4x + 213.5$	0.999	0.004	0.01	10%

[*] SRM transitions defined by analogy to the acetal derivative of oleacein.

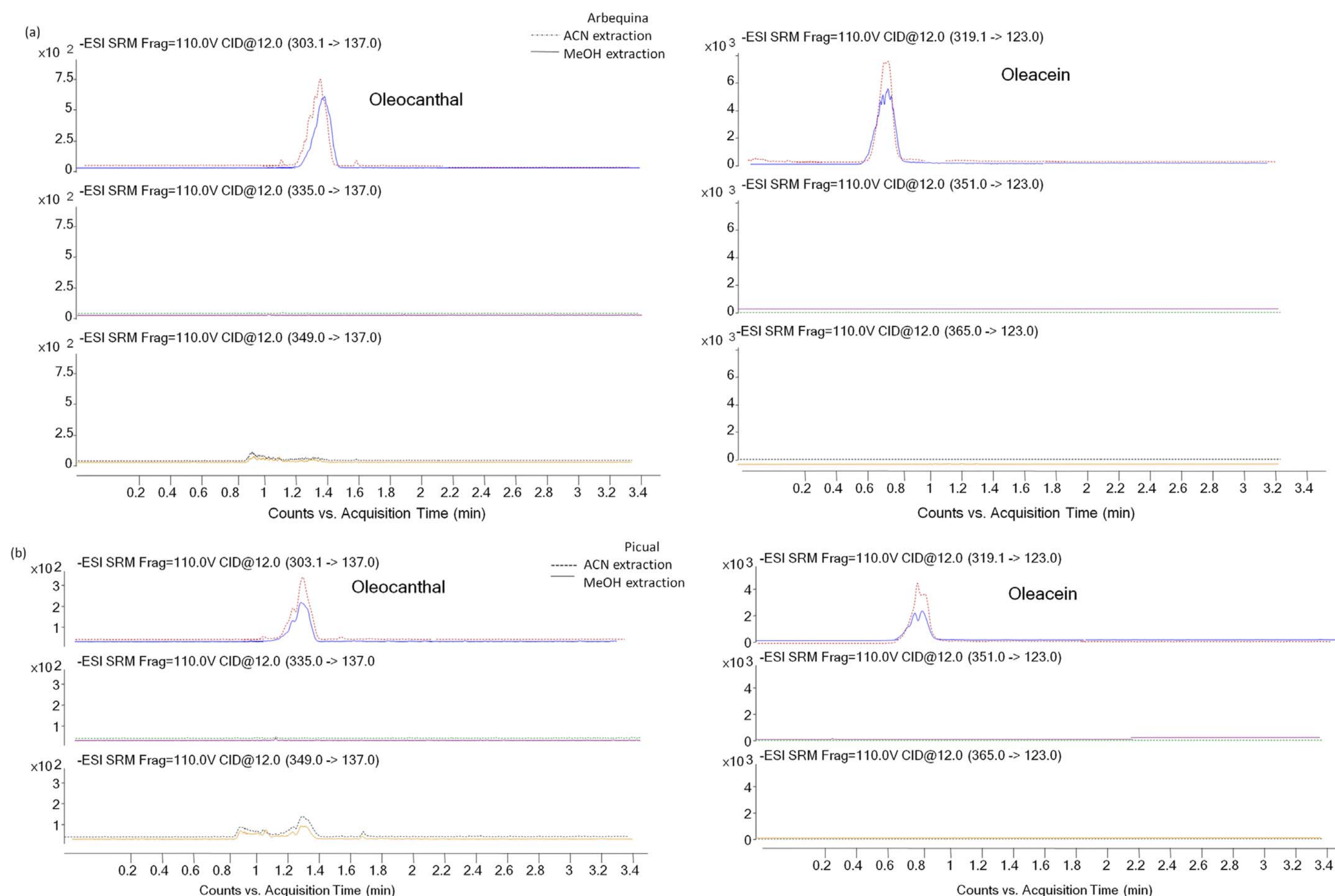

Fig. 3. Chromatograms obtained in selected reaction monitoring mode from analysis of MeOH–water and ACN extracts from (a) Arbequina and (b) Picual VOOs using the ACN chromatographic gradient.

± 1.0% in terms of concentration. However, the dimethyl acetal form of oleocanthal was not detected in any of the extracts from the target monovarietal VOOs. The low conversion rate clearly shows that the use of MeOH gradients in the chromatographic separation should not be discarded since oleocanthal and oleacein could be accurately quantified. In addition, the quantitative response observed for the two phenols was clearly higher in MeOH-based chromatographic gradients than in those using ACN (Supplementary Fig. 4) and the chromatographic resolution was clearly better with the former gradient.

It is worth mentioning that a *t*-test analysis (p-value < 0.05) revealed that no statistically significant differences on the percentages were observed by using MeOH–water or ACN as extractants for the two tested chromatographic methods, as shows Table 2. According to this result, the analysis of phenolic profiles should be carried out after extraction with MeOH–water mixtures due to the variability in the polar character of single phenols. On the other hand, if the determination is targeted at secoiridoids, extraction with ACN constitutes the best strategy because the interferences from compounds more polar than the target analytes would be avoided.

3.4. Quantitative determination of oleocanthal and oleacein in VOO samples

Once proved that the formation of acetal and hemiacetal derivatives is not kinetically favored under the experimental conditions described above, quantitative analysis of oleocanthal and oleacein in three VOO samples was planned. For this purpose, the protocol based on phenol extraction with ACN was applied, while the LC–QqQ MS/MS analysis was based on the MeOH gradient due to the ionization efficiency of the target phenols, higher in the MeOH phase than in ACN medium. Absolute quantitation was performed by using the calibration models prepared with oleocanthal and oleacein standards spiked in refined oil

Table 2
Relative concentration (expressed as percentage) of oleocanthal, oleacein and their hemiacetals and acetals as an average (n=3) of the target VOOs as a function of the chromatographic method.

Compound	MeOH mobile phase		ACN mobile phase	
	Extraction with MeOH–water	Extraction with ACN	Extraction with MeOH–water	Extraction with ACN
Oleacein	94.2 ± 0.5	93.9 ± 0.2	100	100
Methyl hemiacetal of oleacein	5.2 ± 0.4	5.1 ± 0.3	0	0
Dimethyl acetal of oleacein	0.6 ± 0.1	1.0 ± 0.2	0	0
Oleocanthal	90.2 ± 1.5	90.2 ± 1.0	100	100
Methyl hemiacetal from oleocanthal	9.8 ± 1.5	9.8 ± 1.0	0	0
Dimethyl acetal of oleocanthal	0	0	0	0

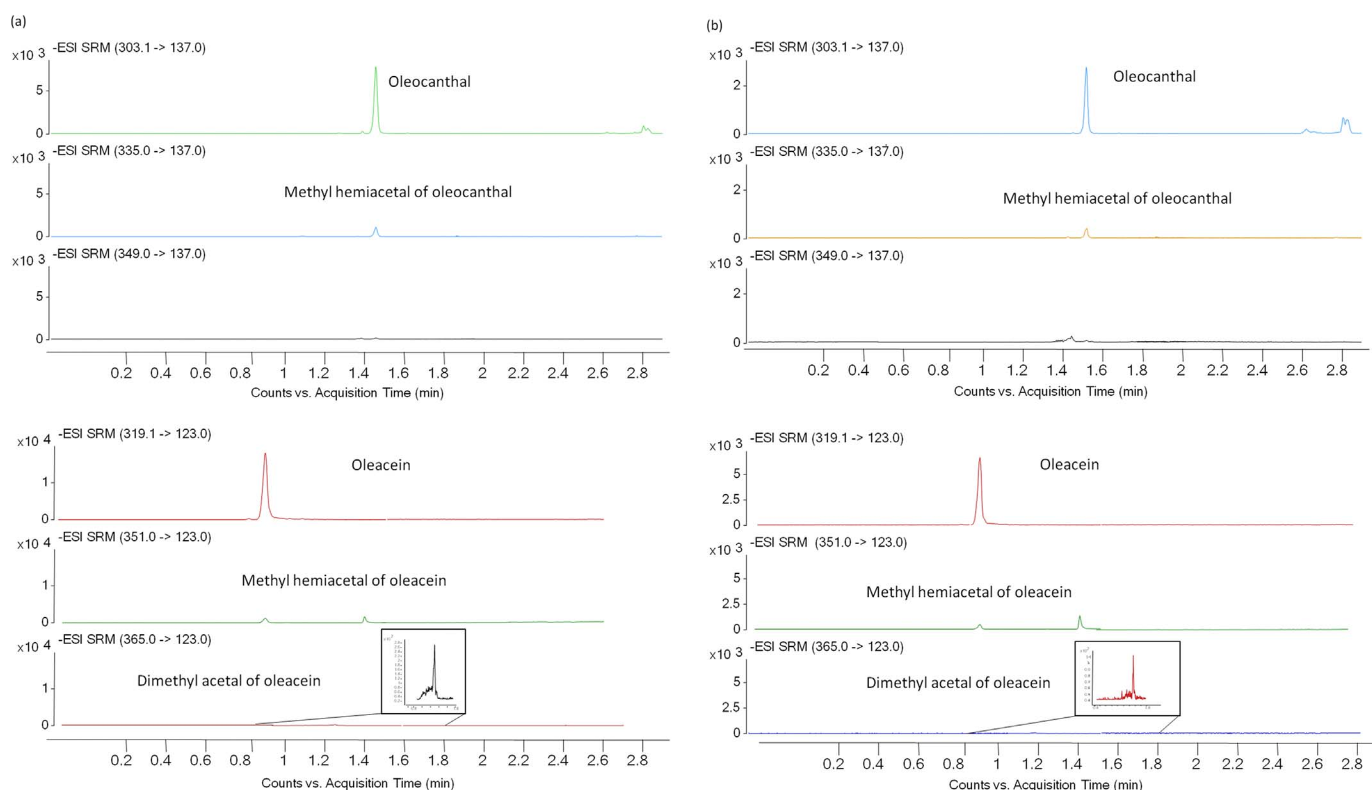

Fig. 4. Chromatograms obtained in selected reaction monitoring mode from LC–QqQ MS/MS analysis of ACN extracts from (a) Picual and (b) Arbequina monovarietal VOOs using a MeOH-based gradient.

(see Table 1). The analyses (n=3) showed that Greek Lianolia Kerkiras VOO contained high concentration of oleocanthal and oleacein (with 537 ± 59 and 392 ± 47 µg/g, respectively). Concerning the two monovarietal VOOs obtained from the two typical Spanish varieties, Picual led to the intermediate levels of oleocanthal with 153 ± 17 µg/g as compared to Arbequina VOO with 67 ± 7 µg/g, while these VOOs provided similar levels of oleacein with 69 ± 8 and 63 ± 7 µg/g for Picual and Arbequina, respectively. It is worth mentioning that a comparison among monovarietal VOOs is not a pursued aim of this research since it is well-known that the concentration of phenols is strongly dependent on several factors, apart from genotype, such as climatic, growing location, fruit ripening, agronomic factors, and mechanical extraction system. As emphasized above, these three monovarietal VOOs were selected according to their content in oleocanthal and oleacein supported on the results cited in the literature.

4. Conclusions

Attending to the results obtained in this study, LC–QqQ MS/MS can be used for quantitative analysis of oleocanthal and oleacein in VOO samples under the conditions described in this research as the conversion of these phenols to acetal and hemiacetal derivatives is very low. The high sensitivity and selectivity levels of SRM makes LC–QqQ MS/MS a competitive technique for analysis of these two phenolic compounds with bioactive properties.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2016.09.056.

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