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Evaluation of Phenolic Compounds in Virgin Olive Oil by Direct Injection in High-Performance Liquid Chromatography with Fluorometric Detection

ROBERTO SELVAGGINI, MAURIZIO SERVILI,* STEFANIA URBANI, SONIA ESPOSTO, AGNESE TATICCHI, AND GIANFRANCESCO MONTEDORO

Dipartimento di Scienze Economico, Estimative e degli Alimenti, Sezione di Tecnologie e Biotecnologie degli Alimenti, Università degli Studi di Perugia, Via S. Costanzo, 06126 Perugia, Italy

Hydrophilic phenols are the most abundant natural antioxidants of virgin olive oil (VOO), in which tocopherols and carotenes are also present. The prevalent classes of hydrophilic phenols found in VOO are phenyl alcohols, phenolic acids, secoiridoids such as the dialdehydic form of decarboxymethyl elenolic acid linked to (3,4-dihydroxyphenyl)ethanol or (*p*-hydroxypheny1)ethanol (3,4-DHPEA-EDA or *p*-HPEA-EDA) and an isomer of the oleuropein aglycon (3,4-DHPEA-EA), lignans such as (+)-1-acetoxypinoresinol and (+)-pinoresinol, and flavonoids. A new method for the analysis of VOO hydrophilic phenols by direct injection in high-performance liquid chromatography (HPLC) with the use of a fluorescence detector (FLD) has been proposed and compared with the traditional liquid−liquid extraction technique followed by the HPLC analysis utilizing a diode array detector (DAD) and a FLD. Results show that the most important classes of phenolic compounds occurring in VOO can be evaluated using HPLC direct injection. The efficiency of the new method, as compared to the liquid−liquid extraction, was higher to quantify phenyl alcohols, lignans, and 3,4-DHPEA-EA and lower for the evaluation of 3,4-DHPEA-EDA and *p*-HPEA-EDA.

KEYWORDS: Phenols; secoiridoids; lignans; virgin olive oil; HPLC; fluorescence detector; DAD

INTRODUCTION

Virgin olive oil (VOO) contains different classes of phenolic compounds such as phenolic acids, phenolic alcohols, hydroxy-isochromans, secoiridoids, lignans, and flavonoids that affect its sensory and healthy properties. The phenolic acids, phenolic alcohols that include the (3,4-dihydroxyphenyl)ethanol (3,4-DHPEA or hydroxytyrosol) and (*p*-hydroxyphenyl)ethanol (*p*-HPEA or tyrosol), hydroxy-isochromans, and flavonoids (*1*) are present in small amounts in VOO (*2*−*4*) while secoiridoids and lignans are the most concentrated phenolic compounds of this oil. The most abundant secoiridoids of VOO are the dialdehydic form of decarboxymethyl elenolic acid linked to (3,4-dihydroxy-phenyl)ethanol or (*p*-hydroxypheny1)ethanol (3,4-DHPEA-EDA or *p*-HPEA-EDA) and an isomer of the oleuropein aglycon (3,4-DHPEA-EA) (*5*−*11*). Oleuropein (*12*), oleuropein and ligstroside aglycons, and their dialdehydic forms (*11*, *13*) were also detected. Flavonoids such as luteolin and apigenin were also reported as phenolic components of VOO by Rovellini et al. (*14*). The last group of phenols found in VOO are the lignans; Owen et al. (*11*, *15*) and Brenes et al. (*16*, *17*), in fact, have recently isolated and characterized (+)-1-acetoxypinoresinol and (+)-pinoresinol as the most concentrated lignans in Italian and Spanish VOOs, respectively.

During the last 15 years, many papers on the evaluation of VOO phenolics by high-performance liquid chromatography (HPLC) have been published (*6*, *11*, *17*−*22*). The main differences among the developed methods can be summarized in the separation procedure of phenols from the oily matrix and the detector choice for the HPLC evaluation.

Concerning the extraction process, two main techniques are reported in the literature: liquid−liquid extraction (LLE) (*4*, *6*, *17*, *19*−*22*) and solid-phase extraction (SPE) (*23*−*27*). Recently, the use of *N*,*N*-dimethylformamide (DMF) in LLE has been proposed by Brenes et al. to reduce the sample manipulation and, at the same time, to improve the recovery efficiency (*17*, *27*).

As regards the qualitative and quantitative evaluation of VOO phenols, many papers were published on the use of HPLC coupled to ultraviolet (UV) detection (*6*, *11*, *19*−*22*, *25*, *26*, *28*); however, electrochemical (EC) and fluorescence detectors (FLDs) were also proposed (*17*, *22*, *23*, *27*). Between the UV detectors, the diode array (DAD) is the most used, also for routine analysis, due to the possibility of this detector to obtain online the UV spectra, which can be very useful to identify the specific substances of the phenolic compounds separated by the HPLC column (*6*, *22*). This aspect is particularly important in the analytical evaluation of VOO phenols due to the difficulty of finding appropriate standards available in commerce. Only the phenolic acids can be found as commercial standards; on

* To whom correspondence should be addressed. Tel: +39 075 5857942. Fax: +39 075 5857916. E-mail: servimau@unipg.it.

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the contrary, secoiridoid derivatives and lignans could be preventively extracted from the oil using semipreparative columns. The use of an EC detector has been proposed for the fist time by Mannino et al. (*23*) to evaluate simple phenols of VOO. Tsimidou et al. (*22*) compared an UV detector, DAD, and EC detector and concluded that the EC detector could be very useful to evaluate phenols occurring in minor amounts due to the high sensibility of the EC detector in comparison to the UV detector and the DAD. Recently, Brenes et al. (*27*) have proposed a rapid analytical methodology for determining phenols concentration in VOO based on coupling the use of DMF in LLE and EC detector in HPLC analysis.

The utilization of fluorometric detection in the analysis of phenolic acids of VOO was proposed by Cartoni et al. (*29*). Brenes et al. compared the EC, UV, and FLD and gas chromatography with mass spectrometer (GC-MS) in the evaluation of several phenolic compounds of VOO and concluded that the FLD was very interesting in the evaluation of lignans for the routine analysis of VOO phenols because they considered it easier to utilize this detector in comparison with GC-MS to discriminate (+)-pinoresinol and (+)-1-acetoxypinoresinol (*27*).

In this paper, a new method for evaluating the hydrophilic phenols of VOO, based on the direct injection (DI) in HPLC with the use of a FLD, has been proposed and compared with the traditional LLE. A comparison between fluorescence and DADs has also been reported.

MATERIALS AND METHODS

VOOs. VOO samples from Italy, Spain, Israel, Egypt, Argentina, and Chile were collected. The Italians oils were extracted by centrifugation using a decanter Rapanelli model 400 ECO/G, at a low level of water addition, from the following cultivars: Coratina, Frantoio, Canino, Leccino, Moraiolo, and Cornicabra.

Solvents and Reference Compounds. For LLE and HPLC analyses, the solvents methyl alcohol, *n*-hexane, acetonitrile, and acetone, all of HPLC grade, and glacial acetic acid were used and were obtained from Carlo Erba (Milan, Italy). Water of both analytical and HPLC grade was obtained by using purification units. The dialdehydic form of elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA and *p*-HPEA-EDA, respectively), the isomer of oleuropein aglycon (3,4-DHPEA-EA), (+)-1-acetoxypinoresinol, and (+)-pinoresinol were extracted from VOO and separated by semipreparative HPLC according to the procedure reported previously (*8*). The nuclear magnetic resonance (NMR) data of 3,4-DHPEA-EDA, *p*-HPEA-EDA, and 3,4-DHPEA-EA were consistent with those reported by Montedoro ed al. (*8*), while the NMR data relative to (+)-1-acetoxypinoresinol and (+)-pinoresinol were in accordance with those obtained by Owen et al. (*15*). The 3,4-(dihydroxyphenyl)ethanol (3,4-DHPEA) was obtained from Cabru S.p.A. (Milan, Italy), and *p*-HPEA was purchased from Fluka (Milan, Italy).

Sample Preparations and HPLC Analysis. *LLE Process.* The LLE was performed according to Montedoro et al. (*6*). Before injection in HPLC, the phenolic extract was solubilized with 1 mL of methanol and filtered through a polyvinylidene fluoride (PVDF) syringe filter (0.2 μm).

DI of VOO. For DI of VOO, 2 g of oil was dissolved in 10 mL of acetone and then this solution was filtered through a PVDF syringe filter (0.2 μm).

Reversed Phase HPLC Analysis. The HPLC analysis was conducted with an Agilent Technologies system model 1100 composed of a vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment, a DAD, and a FLD. The analysis of the oil extract was performed using the following procedure.

The C18 columns used were a ChromSep Inertsil ODS-3 250 mm $\times$ 4.6 mm column with a particle size of 5 μm with a ChromSep guard column 100 mm $\times$ 3.0 mm (Varian Inc., Walnut Creek, CA) and a Spherisorb ODS-1 250 mm $\times$ 4.6 mm columns with a particle size of 5 μm (Phase Separation Ltd., Deeside, United Kingdom). The injected sample volumes for both VOO methanolic extracts and VOO DIs were 20 μL.

The two columns were operated with the same mobile phase that was composed of 0.2% acetic acid (pH 3.1) in water (solvent A)/methanol (solvent B) at a flow rate of 1.5 mL/min when the ChromSep Inertsil ODS-3 column was used and 1 mL/min when the Spherisorb ODS-1 was utilized; for both columns, the gradient changed as follows: 95% A/5% B for 2 min, 75% A/25% B in 8 min, 60% A/40% B in 10 min, 50% A/50% B in 16 min, and 0% A/100% B in 14 min; this composition was maintained for 10 min, then returned to initial conditions and equilibration in 13 min; the total running time was 73 min.

The wavelength used for the detection with DAD was 278 nm; instead, the FLD was operated at an excitation wavelength set at 280 nm and emissions at 313, 339, 353, and 453 nm. The photomultiplier (PMT) gain of the FLD was set to eight for the oil extract analysis and to 13 for DI to increase its sensitivity. The acquisition of chromatographic data was done during 60 min from the beginning of the analysis.

Statistical Analysis. For comparing results obtained and to test for significative differences between the two detectors and between the two methods, the *t*-test was employed using SigmaStat software v. 2.0.

RESULTS AND DISCUSSION

Columns and Detectors Comparisons. Two stationary phases were tested on the effectiveness of peak separations of VOO phenols present in the phenolic extract. **Figure 1**, reporting the HPLC chromatograms of standard compounds and a VOO phenolic extract, obtained from cv. Canino VOO, obtained using DAD, shows a different efficiency in the peak separation operated by the two columns. In particular, as indicated in the HPLC chromatogram relative to the standard compounds, the ODS-3 column shows a poor resolution of the peaks corresponding to *p*-HPEA-EDA and (+)-pinoresinol and, at the same time, splits the peaks corresponding to 3,4-DHPEA-EDA, *p*-HPEA-EDA, and 3,4-DHPEA-EA. The peak splitting observed for these compounds may be due to the methanolic addition to the aldehydic groups, occurring in these substances, as highlighted in a previous paper (*8*). The results related to the methanolic extract confirm the lower efficiency of ODS-3 in the separation of *p*-HPEA-EDA and (+)-pinoresinol, which, in this case, are nearly completely overlapped. However, this column shows a higher selectivity in the region where the peaks elute corresponding to 3,4-DHPEA-EDA and *p*-HPEA-EDA, thus permitting a better resolution of the different forms of oleuropein and ligstroside aglycons found by Rovellini et al. (*13*). The ODS-1 column, on the contrary, shows a better separation of the peaks corresponding to *p*-HPEA-EDA, (+)-1-acetoxypinoresinol, and (+)-pinoresinol that now are nearly completely separated. In addition, comparing the behavior of the two columns, an inversion of the elution order of the two lignans is also observed.

Even modifying the mobile phase composition, the results obtained with ODS-3, particularly in the region where *p*-HPEA-EDA and lignans elute, the peak separations are not enhanced (data not shown). Concerning the phenolic alcohols, the results obtained using standard solutions and a VOO methanolic extract show a similar selectivity of the two columns.

In terms of detector comparison between DAD and FLD, results reported in **Figure 2**, which exhibits the chromatograms corresponding to the standard solution and the VOO methanolic extract from cv. Moraiolo, using the ODS-1 stationary phase, prove that the FLD has a higher height of peaks corresponding to the phenyl alcohols, lignans, and 3,4-DHPEA-EA, while a

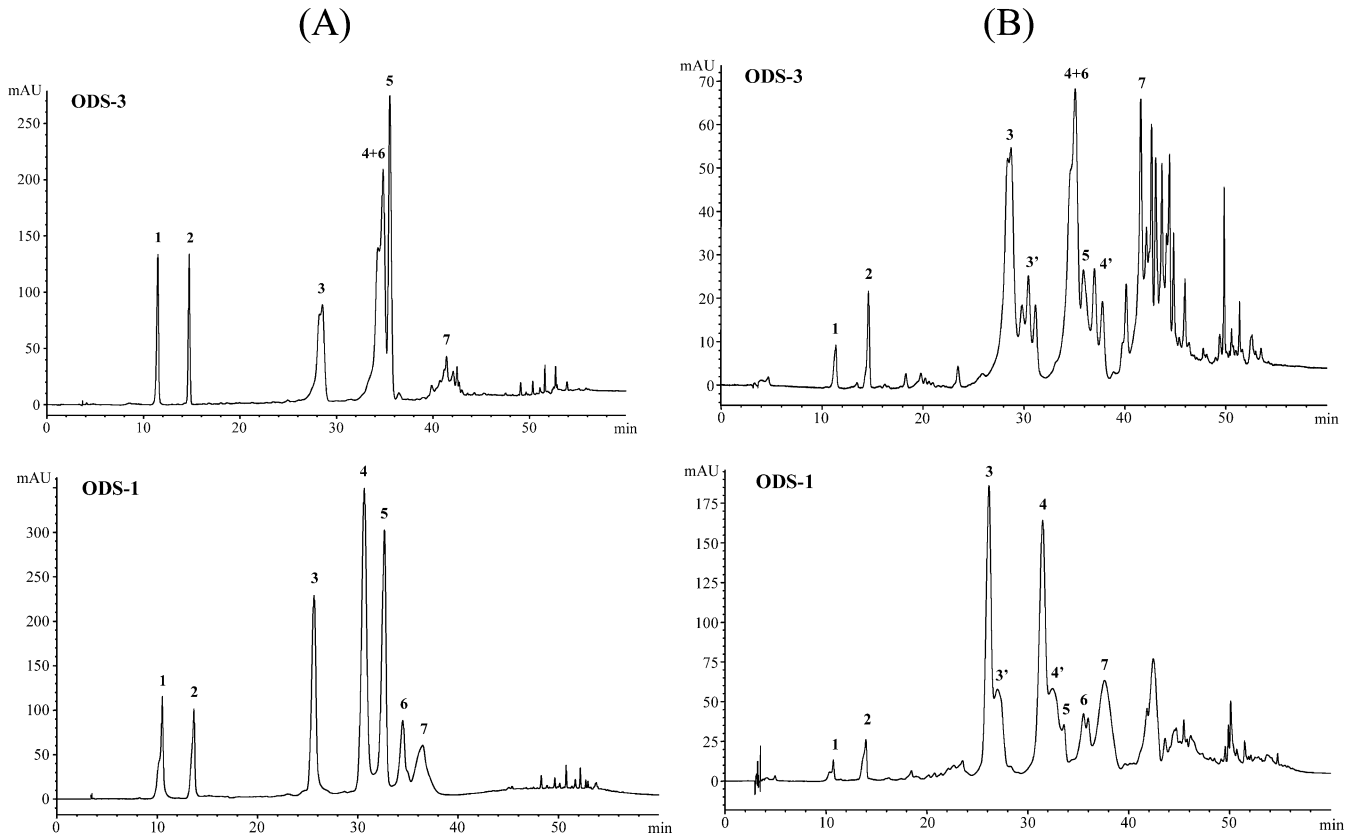

Figure 1. HPLC chromatograms of standards compounds (**A**) and VOO methanolic extract of cv. Canino (**B**) obtained using the ODS-3 and ODS-1 stationary phases and recorded with DAD at 278 nm. Peak numbers: 1, 3,4-DHPEA; 2, *p*-HPEA; 3, 3,4-DHPEA-EDA; 4, *p*-HPEA-EDA; 5, (+)-1-acetoxypinoresinol; 6, (+)-pinoresinol; and 7, 3,4-DHPEA-EA [3′ and 4′ structures identified by Rovellini et al. (*14*)].

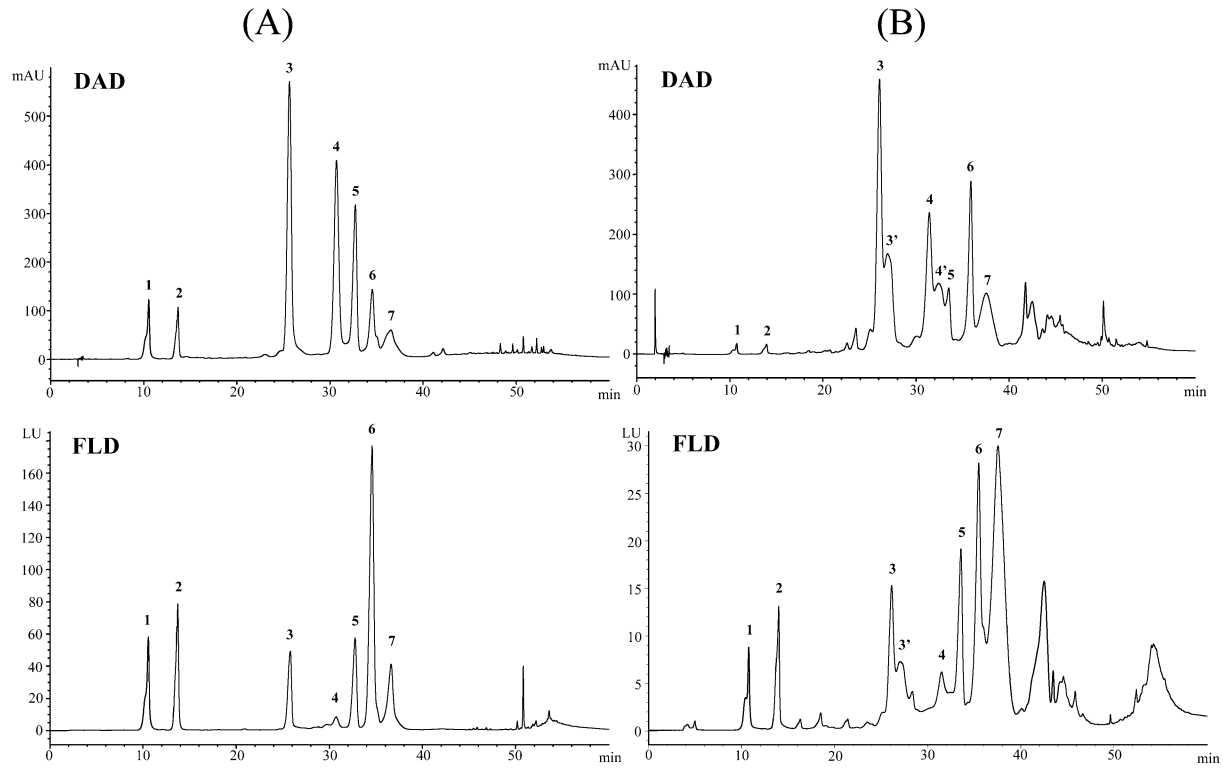

Figure 2. HPLC chromatograms of standards compounds (**A**) and VOO methanolic extract of cv. Moraiolo (**B**) recorded with DAD at 278 nm and FLD set at ex = 280 nm and em = 339 nm. For peak numbers, refer to **Figure 1**.

lower detector response was observed for 3,4-DHPEA-EDA and *p*-HPEA-EDA. This behavior is due to the chemical structures of the last two compounds that contain a dialdehydic group in the open elenolic acid ring. This structure, producing a minor planarity of the molecules, gives, as a consequence, a reduced fluorescence intensity.

The multiemission mode was applied in the fluorimetric evaluation. In fact, as shown in **Figure 3**, reporting the HPLC

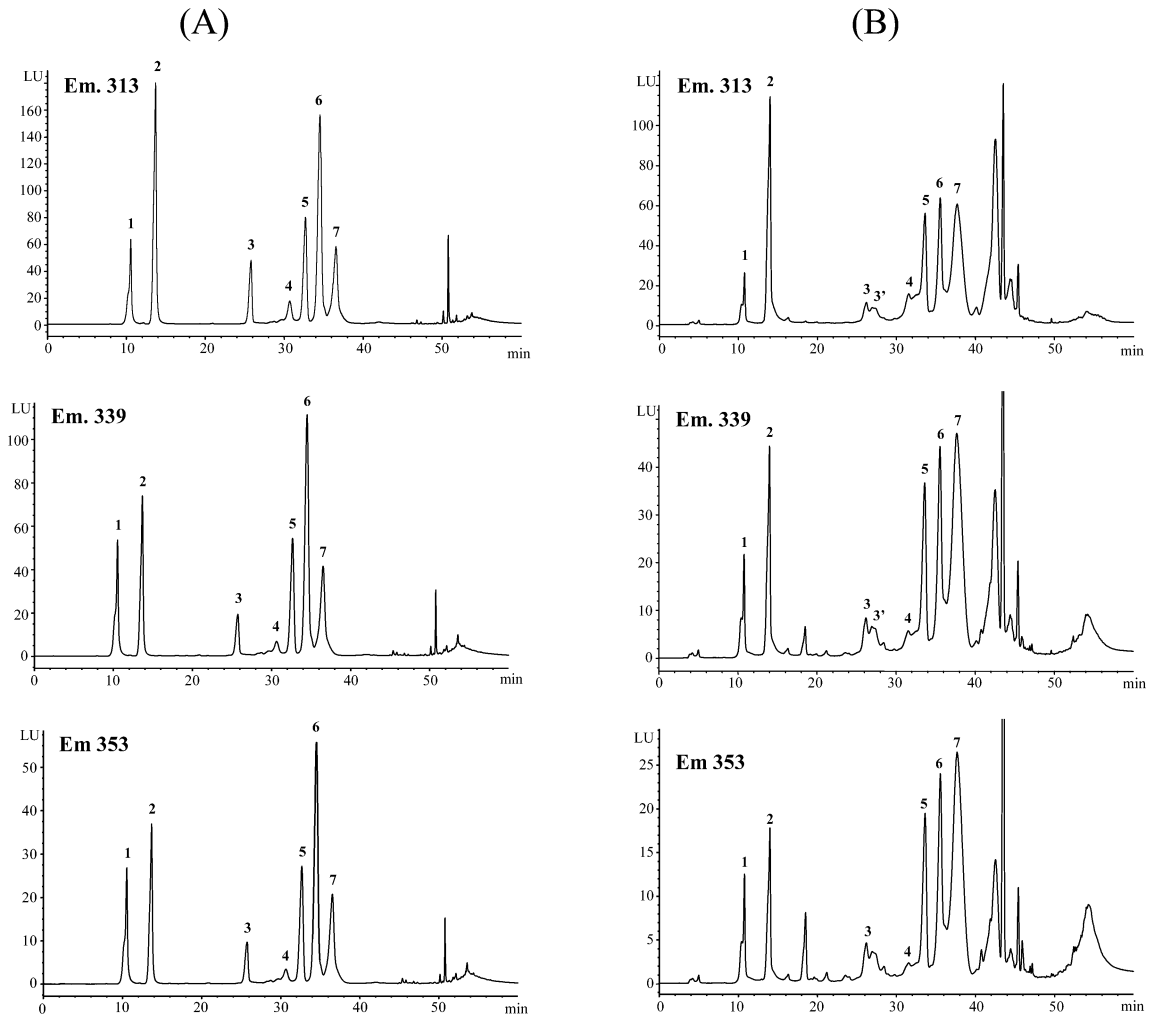

Figure 3. HPLC chromatograms of standards compounds (**A**) and VOO methanolic extract of cv. Coratina (**B**) using FLD set at ex = 280 nm and em = 313, 339, and 353 nm. For peak numbers, refer to **Figure 1**.

chromatograms obtained at three different emission wavelengths of standard solution and VOO phenolic extract, from cv. Coratina, using the ODS-1 column, the different optimal conditions found for the qualitative and quantitative analysis of the phenyl alcohols, secoiridoid derivatives, and lignans were different. In particular, while the excitation wavelength was fixed at 280 nm for all of the compounds, the emission wavelength was set to 313 nm for *p*-HPEA-EDA, 339 nm for the (+)-1-acetoxypinoresinol and (+)-pinoresinol, and 353 nm for 3,4-DHPEA, *p*-HPEA, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA. Concerning the phenolic acids, such as vanillic and *p*-coumaric acids, which have not been evaluated in the present work, their quantitation can always be made with an excitation of 280 nm and with the emission set at 353 nm for the former and 453 nm for the latter.

A preliminary comparative quantitative study to evaluate the detector responses was made by using the Spherisorb ODS-1 stationary phase and constructing calibration curves with standard compounds to both verify the linearity of response of FLD and to determine the quantitation limits (QLs) of DAD and FLD, considering a signal-to-noise ratio of 10 according to the ACS convention. The result of **Table 1** points out the good linearity of FLD in the range of concentrations greater than those used to evaluate phenolic concentration in VOO and, at the same time, shows very low QLs of the FLD, confirming the higher sensibility of this detector with respect to DAD.

Table 1. Linearity and Detection Limits Determined with DAD and FLD with Reference Compounds[a]

compound	concentration range (mg/L)	R^2 (FLD)	QL (μg/kg) DAD	QL (μg/kg) FLD
3,4-DHPEA (i)	0.3–30	0.997	350	85
p -HPEA (i)	0.6–53	0.997	315	100
3,4-DHPEA-EDA (i)	20–1100	0.998	1000	3500
p-HPEA-EDA (ii)	5–600	0.998	1100	850
(+)-1-acethoxypinoresinol (iii)	5–700	0.997	200	40
(+)-pinoresinol (iii)	5–700	0.998	250	150
3,4-DHPEA-EA (i)	20–800	0.998	1800	600

[a] The phenolic composition was determined with DAD at 278 nm and with FLD at different wavelengths: (i) ex = 280 nm and em = 353nm; (ii) ex = 280 nm and em = 313 nm; and (iii) ex = 280 nm and em = 339 nm.

The quantitative analysis, obtained using the two detectors with the Spherisorb ODS-1 column, of the VOO methanolic extracts from various Italian cultivars is reported in **Table 2**. The concentrations measured with the FLD and DAD put in evidence low differences but are statistically significant for several compounds that include phenyl alcohols, oleuropein, and ligstroside derivatives such as 3,4-DHPEA-EDA, 3,4-DHPEA-EA, and *p*-HPEA-EDA. On the contrary, the quantification of lignans shows a different behavior; in fact, mainly the concentrations of the (+)-pinoresinol measured with the FLD are very high in comparison to the DAD values. The use of the ODS-3

Table 2. Phenolic Compounds Concentration in VOO: Comparison between DAD and FLD Evaluated on the Methanolic Extract (mg/kg) Using the ODS-1 Column[a]

	cultivar					
	Coratina		Canino		Moraiolo	
compound	DAD	FLD	DAD	FLD	DAD	FLD
3,4-DHPEA (i)	4.0 (0.1) a	4.3 (0.1) a	1.1 (0.05) a	1.3 (0.03) b	1.5 (0.02) a	1.5 (0.004) a
p -HPEA (i)	5.8 (0.1) a	6.3 (0.04) b	2.6 (0.01) a	2.7 (0.02) b	2.2 (0.004) a	2.5 (0.1) b
3,4-DHPEA-EDA (i)	230.2 (1.5) a	216.4 (2.8) b	111.2 (0.4) a	105.0 (1.4) b	285.1 (0.7) a	273.0 (4.4) b
p-HPEA-EDA (ii)	72.1 (0.2) a	80.0 (2.9) b	36.2 (0.04) a	39.1 (0.1) b	93.2 (0.2) a	109.7 (0.4) b
(+)-1-acethoxypinoresinol (iii)	13.7 (0.03) a	22.6 (0.2) b	2.0 (0.1) a	2.9 (0.02) b	8.3 (0.01) a	11.5 (0.2) b
(+)-pinoresinol (iii)	7.0 (0.01) a	34.1 (0.3) b	5.2 (0.1) a	27.4 (0.1) b	27.4 (0.03) a	23.9 (0.2) b
3,4-DHPEA-EA (i)	221.7 (0.5) a	223.8 (3.6) a	81.2 (0.1) a	86.2 (0.3) b	139.4 (0.7) a	141.1 (0.1) a

[a] Results are mean values of three replicates; numbers in parentheses represent ± standard deviations. Values in each row relative to the two detectors for each cultivar bearing the same superscripts are not significantly ($P < 0.01$) different from one another. For compound name's superscripts, see **Table 1**.

column gave similar results, with the exception of *p*-HPEA-EDA and (+)-pinoresinol (data not shown). However, the differences of the values determined with the two detectors are not constant and they are lower in cv. Moraiolo and higher in cv. Coratina, pointing out that the genetic origin of VOO could affect them. An HPLC analysis performed on refined olive oil, to show the potential matrix effect that may interfere with the detector response, did not show any interference in the chromatographic region where the phenolic compounds studied in this work elute. The potential effect of α-tocopherol in the FLD response was also evaluated, but this compound, with the chromatographic conditions chosen for the hydrophilic phenols analysis, elutes at a retention time of 55.5 min and consequently far away from the lignans chromatographic region. These results, which need further investigation, utilizing different columns, may be related to the occurrence, in the chromatogram region where the lignans elute, of unknown compounds overlapped with (+)-pinoresinol that should have a great fluorescence intensity and a low UV absorbance.

Comparison between LLE and DI. Figure 4 shows a comparison between the HPLC chromatograms of phenolic compounds of VOO from cv. Frantoio, obtained using the ODS-1 column, and FLD relative to LLE and DI methods. Results show a very similar peak distribution and separation efficiency between LLE and DI, indicating that the introduction of the oil directly into the column does not modify its performance and does not shorten its natural life. However, when the oil is directly injected, it is necessary to run a blank analysis every six injections to perform a better cleaning of the column so as to maintain their performances and avoid peak broadening. To estimate the precision of the new method, the repeatability has been determined to establish the intraday and interday variability measuring the coefficient of variation (CV) of each compound by injecting six times the same oil and repeating the same operations 2 days consecutively using FLD. The CV values reported in **Table 3** show very good intraday variability (all below or equal to 1%) and have been found for phenyl alcohols, lignans, and 3,4-DHPEA-EA; good CV values for 3,4-DHPEA-EDA and *p*-HPEA-EDA (better than 3%) have been found. The interday variability was more unfavorable, however, remaining below 2.9% for all compounds with the exception of *p*-HPEA-EDA. Another important observation arising from **Table 3** is that the intraday and interday variability are almost the same for LLE and DI, denoting that the oily matrix does not interfere with analysis. Analyses of VOO by DI were also performed using the ODS-3 column, and the results do not put in evidence qualitative differences with LLE, confirming the possibility of utilization of this column even for DI (data not shown).

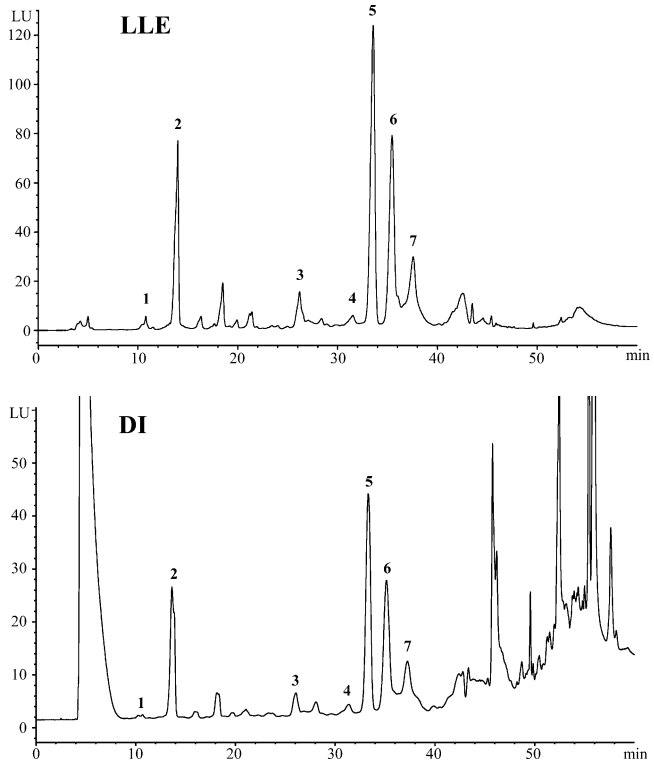

Figure 4. HPLC chromatograms of VOO, cv. Frantoio, obtained by LLE methanolic extract and DI using FLD signal set at ex = 280 nm and em = 339 nm. For peak numbers, refer to **Figure 1**.

Table 3. Repeatability (Intraday and Interday) Expressed as CV of the Analyses of the Phenolic Compounds Present in a VOO Determined with the Two Methods Using FLD[a]

	LLE CV		DI CV	
compound	intraday	interday	intraday	interday
3,4-DHPEA (i)	0.7	0.9	0.7	1.0
p-HPEA (i)	0.8	2.9	0.8	2.8
3,4-DHPEA-EDA (i)	1.4	1.6	1.4	1.8
p-HPEA-EDA (ii)	2.7	3.3	2.3	5.0
(+)-1-acethoxypinoresinol (iii)	0.8	1.7	0.6	1.9
(+)-pinoresinol (iii)	0.6	0.5	0.5	0.5
3,4-DHPEA-EA (i)	1.0	2.4	0.9	2.3

[a] For compound name's superscripts, see **Table 1**.

The quantitative results reported in **Table 4**, obtained comparing the two methods using the same fluorimetric detector and the same ODS-1 column, show that the DI provides higher values for the most important phenolic compound in comparison

Table 4. Phenolic Compounds (mg/kg) Evaluation in VOO: Comparison between LLE and DI Using FLD[a]

	cultivar					
	Cornicabra		Leccino		Frantoio	
compound	LLE	DI	LLE	DI	LLE	DI
3,4-DHPEA (i)	11.9 (0.01) a	19.0 (0.3) b	1.9 (0.9) a	2.8 (0.1) a	0.3 (0.02) a	0.4 (0.05) a
p-HPEA (i)	8.8 (0.02) a	18.1 (0.1) b	6.9 (0.02) a	7.2 (0.02) b	10.1 (0.2) a	11.7 (0.03) b
3,4-DHPEA-EDA (i)	86.2 (0.3) a	114.2 (2.1) b	184.3 (4.8) a	196.1 (0.03) b	136.5 (2.1) a	158.1 (3.7) b
p-HPEA-EDA (ii)	50.8 (0.2) a	79.6 (1.2) b	58.2 (1.5) a	94.0 (0.2) b	78.2 (0.6) a	82.8 (0.3) b
(+)-1-acethoxypinoresinol (iii)	2.6 (0.3) a	4.9 (0.2) b	34.2 (0.2) a	72.8 (1.7) b	56.3 (0.4) a	58.1 (0.1) b
(+)-pinoresinol (iii)	33.9 (0.5) a	74.5 (0.4) b	36.1 (0.3) a	53.2 (0.4) b	54.4 (0.4) a	55.1 (0.1) b
3,4-DHPEA-EA (i)	117.7 (0.4) a	215.8 (0.8) b	185.8 (0.8) a	204.2 (0.9) b	98.3 (0.3) a	121.3 (1.6) b

[a] The phenolic content is the mean value of four independent experiments; numbers in parentheses represent ± standard deviation. Values in each row relative to the two methods for each cultivar bearing the same superscripts are not significantly ($P < 0.01$) different from one another. For compound name's superscripts, see **Table 1**.

Table 5. QLs of the Phenolic Compounds Evaluated on VOO by LLE and DI with FLD (μg/kg)[a]

compound	LLE QL	DI QL
3,4-DHPEA (i)	9	100
p-HPEA (i)	12	150
3,4-DHPEA-EDA (i)	550	4000
p-HPEA-EDA (ii)	300	2500
(+)-1-acethoxypinoresinol (iii)	5	50
(+)-pinoresinol (iii)	16	200
3,4-DHPEA-EA (i)	300	2900

[a] For compound name's superscripts, see **Table 1**.

with the LLE. The highest differences were observed for cvs. Cornicabra and Leccino, mainly for the secoiridoids derivatives and the lignans. As observed by Brenes et al. (*27*), comparing two LLE methods, performed using methanol/water (80/20% v/v), according to Montedoro et al. (*6*), and DMF, the extraction procedures produce a partial and selective recovery of VOO phenolic compounds; this aspect is due to the different polarities, structures, and molecular weights of the various classes of VOO phenols. In addition, phenols can be partially oxidized during sample preparation. All of these aspects can explain the differences between the LLE and the DI of oil in the VOO phenols evaluation.

The QLs of the phenolic compounds obtained using LLE and DI employing for both cases FLD have been determined, always considering a signal-to-noise ratio of 10 according to the ACS convention. Results summarized in **Table 5** show a very low QL with both LLE and DI methods for several compounds such as lignans, 3,4-DHPEA, and *p*-HPEA; the detection limits for secoiridoids were higher but in any case much lower than the concentrations generally found in VOOs. This is an important result that confirms the potentiality of the DI for the VOO hydrophilic phenols evaluation.

Conclusion. This is the first time that an HPLC DI analysis has been proposed to evaluate VOO hydrophilic phenols. The use of DI, involving very low sample manipulation, reduces the risk of artifacts, overcoming the problems related to the extraction, and, consequently, can be proposed to obtain good results in a shorter time. The linearity of response and the sensibility of FLD used for the DI analysis are very good and permit the phenolic compound evaluation in VOO.

Comparing DAD and FLD, some differences have been observed in the quantitation mainly for (+)-pinoresinol, which can probably be due to the coelution of unknown substances present in different amounts in VOO depending on the cultivar. Work is in progress to study this specific aspect.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; VOO, virgin olive oil; 3,4-DHPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to (3,4-dihydroxyphenyl)-ethanol; 3,4-DHPEA-EA, isomer of the oleuropein aglycon; *p*-HPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to (*p*-hydroxypheny1)ethanol; DAD, diode array detector; 3,4-DHPEA, (3,4-dihydroxyphenyl)ethanol; *p*-HPEA, (*p*-hydroxyphenyl)ethanol; LLE, liquid−liquid extraction; SPE, solid-phase extraction; DMF, *N*,*N*-dimethylformamide; UV, ultraviolet; EC, electrochemical; GC-MS, gas chromatography with mass spectrometer; FLD, fluorescence detector; NMR, nuclear magnetic resonance; PVDF, polyvinylidene fluoride; PMT, photomultiplier; QL, quantitation limit; DI, direct injection.

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