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Direct determination of phenolic compounds and phospholipids in virgin olive oil by micellar liquid chromatography

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Abstract

A simple HPLC method is reported for fast separation and determination of phenolic compounds (tyrosol, caffeic acid, *p*-coumaric acid and oleuropeina) and phospholipids (phosphatidylethanolamine and phosphatidylcholine) in virgin olive oil samples. The samples were diluted with 2-propanol and injected into the column directly without previous extraction. Samples with an olive oil content of up to 65% were injected without any problems. The analytes were separated on a C-18 column by a micellar mobile phase containing 0.07 M SDS and 2.5% 2-propanol at pH 3, and were detected at 210 nm. Linear calibration curves [$r^2 > 0.997$] were obtained with detection limits ranging from 0.052 to 0.16 μg/g and 1 to 8.6% repeatability for the phenolic compounds. Several virgin olive oil samples were analysed and the recovery values were around 110%.

Keywords: Phenolic compounds; Phospholipids; Micellar liquid chromatography; Direct sample injection; Virgin olive oil

1. Introduction

In recent years, the consumption of foodstuffs rich in antioxidants has been widely recommended. Such is the case of olive oil in the Mediterranean diet, even in non-producing countries like the United States, Australia and Japan. It is well known that some of the compounds found in virgin olive oil–phenols (Decker, 1997; Keceli & Gordon, 2001; Mateos, Dominguez, Espartero, & Cert, 2003; Morgan, Klucas, & Graye, 1997; Ryan & Robards, 1998; Servili et al., 2004; Weinbrenner et al., 2004) and phospholipids (Carelli, Brevedab, & Crapiste, 1997; Singleton & Stikeleather, 1995) – not only affect the sensorial quality of the oil but also have an antioxidant effect. Their ability to bind themselves to the metals present in the oil may inhibit the catalytic activity of the metals and their ability as radical scavengers is also well known. It has also been reported that some of the phospholipids found in vegetable oils display important biological activity (Nomilos, Karantonis, Fragopoulou, & Demopoulos, 2002) and have proved useful in the prevention of certain diseases like arteriosclerosis (Antonopoulou & Karantonis, 2002). The complex-forming capacity of polyphenols and different metals has already been reported in other food samples, for example, Fe, Cu and Zn in wine (Karadjova, Izgi, & Gücer, 2002) and Al in tea samples (Erdemoghu, Pyrzyniska, & Gücer, 2000).

The vast majority of the methods used for separating and determining phenols and phospholipids in vegetable oils involve solid-phase extraction followed by chromatographic techniques. Several reviews and articles dealing with the subject can be found in the literature (Bianco et al., 2003; Carelli et al., 1997; Cert, Moreda, & Pérez-Camino, 2000; Giocometti, Milosevic, & Milin, 2002; Hrncirik & Fritsche, 2004; Lercker & Rodriguez-Estrada, 2000; Nomilos et al., 2002; Panagiotopoulou & Tsimidou, 2002; Ruiz-Gutiérrez & Perez-Camino, 2000; Singleton & Stikeleather, 1995; Thomaidis & Georgiou, 1999). The main drawback of these methods is that they often require complex sample preparation procedures, and in addition

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HPLC is time-consuming technique. Different types of cartridge (C-18, silica, etc.), in which the phenols and phospholipids are retained, and the lipid fraction is eliminated, have been used in solid-phase extraction. The eluted extract is then subjected to the chromatographic process. Capillary electrophoresis has also been used more recently for determining phenolic acids in olive oil (Buiarelli, Di Berardino, Coccioli, Jasionowska, & Russo, 2004; Bonoli, Montanucci, Toschi, & Lercker, 2003). The main advantages of using capillary electrophoresis, even in its basic mode (capillary zone electrophoresis (CZE)) for phenol analysis are its high separation power. Fast and well-resolved separations are obtained for similar compounds, with the possibility of using a low detection wavelength, which leads to high sensitivity. However, although the separation of the different phenolic compounds by CZE (Bonoli et al., 2003) was carried out in 10 min, the real sample also required a previous extraction step.

The possibility of injecting a sample like olive oil directly into the chromatographic column without previous extraction procedures is a very interesting alternative from the point of view of reproducibility, accuracy, lower risk of contamination and it is also less time-consuming. Since it was first proposed as an alternative to conventional reverse-phase chromatography (Armstrong & Nome, 1981), micellar liquid chromatography (MLC) has been used for different types of samples (Capella-Peiró, Gil-Agustí, Monferrer-Pons, & Esteve-Romero, 2002; García Alvarez-Coque & Carda Broch, 1999; Halko & Hutta, 2002; Noguera-Ortí, Villanueva-Camañas, & Ramis-Ramos, 2000) because "dirty samples" can be analysed directly by this technique. In comparison to other hydro-organics eluents, the micellar mobile phases are less flammable, inexpensive, non-toxic and biodegradable. The solubilizing ability of the micelles allows quantification of hydrophilic and hydrophobic analytes in complex matrices. The main drawback of MLC if compared with reverse-phase chromatography is that efficiency is worse, but it improves in the presence of a short-chain alcohol (Khaledi, 1997).

The aim of our work was to optimise the separation by MLC of different phenolic compounds (tyrosol, *p*-coumaric acid, caffeic acid and oleuropeina) and phospholipids (phosphatidylethanolamine (PE) and phosphatidylcholine (PC)), found in virgin olive oil in their natural form, because their ability to bind themselves to the metals, by injecting the sample directly without pretreatment. Separation is performed by using a C18 column and a micellar mobile phase.

2. Experimental

2.1. Instrumentation

A high-resolution liquid chromatograph (Kontron system 400, Kontron Instruments) fitted with two alternating twin piston pumps (model 420), a high pressure mixing chamber (model M-491), an automatic sample injector (50 μl) (model 460) and a detector UV/Vis of variable wavelength (HPLC detector 430, Kontron Instruments) were used. Nucleosil 120 C18 (5 μm, 20×0.46 cm i.d.) (Teknokroma, Barcelona, Spain) was employed as the analytical column. A column (10 μm, 35×4.6 mm i.d.) (Teknokroma, Barcelona, Spain) of the same characteristics was used as the guard column. With regard to the measurement wavelength, although the absorption maximums for tyrosol, *p*-coumaric acid and caffeic acid, and for oleuropeina are 280 and 256 nm, respectively, measurements of both, phenolic compounds and phospholipids, were carried out at 210 nm as a compromise, because 210 nm is the most suitable wavelength for phospholipids and the separation of all the compounds was performed in the same chromatogram (phospholipids are not usually determined by conventional reverse-phase chromatography). Although the wavelength selected is problematic, the baseline obtained was quite satisfactory given the low content of 2-propanol (2.5%) in the mobile phase.

2.2. Reagents, standards and samples

Aqueous solutions were prepared using ultrapure water, with a resistivity of 18.2 MW obtained from a Milli-Q water purification system (Millipore, Saint Quentis Yvelines, France). The following reagents were used: sodium dodecylsulphate (SDS) (pro. Analysi, Merck, Darmstadt, Germany), 2-propanol and 1-butanol HPLC grade, (Lab-Scan, Analytical Science, Dublin, Ireland) for preparation of the micellar mobile phase (Ultrapure bioreagent, J.T. Baker, Deventer, Holland). Acetic acid and ammonium hydroxide (Suprapur, Merck) were used as pH buffering reagents in the mobile phase. Buffer solutions at pH 3 were prepared with hydrochloric acid (pro analysi, Merck) and ammonium dihydrogenphosphate (0.01 M) (Suprapur, Merck), at pH 4.5 with acetic acid and ammonium acetate (Suprapur, Merck) and at pH 6.5 with ammonium dihydrogenphosphate (0.01 M) (Suprapur, Merck). The following solutions were prepared from the standard compounds (Fluka Chemi, Buchs, Switzerland): 1000 μg/g of tyrosol, 1260 μg/g of caffeic acid, 1070 μg/g of *p*-coumaric acid, 2060 μg/g of oleuropeina, 1000 μg/g of phosphatidylethanolamine and 965 μg/g of phosphatidylcholine each one dissolved in 2-propanol. A standard solution prepared by dilution of the solutions, containing tyrosol (125 mg/kg), caffeic acid (150 mg/kg), *p*-coumaric acid (125 mg/kg), oleuropeina (100 mg/kg) phosphatidylethanolamine (100 mg/kg) and phosphatidylcholine (100 mg/kg) in 2-propanol was used for optimisation of the separation of the compounds by MLC. Standards with concentrations ranging from 5 to 150 μg/g of tyrosol, caffeic acid, *p*-coumaric acid, and phosphatidylethanolamine dissolved in 2-propanol were used for the calibration.

Different mobile phases were employed for optimisation of the mobile phase and their compositions and concentrations are given in Table 1.

Table 1
Composition of different mobile phases for optimisation of separation by micellar liquid chromatography

Compound	Concentration											
SDS (M)		0.03			0.05			0.07			0.1	
2-Propanol (%, v/v)	2.5[a]		5	2.5[a]		5	2.5[a]		5	2.5		5
1-Butanol (%, v/v)		–		2.5	5	10	2.5	5	10	2.5	5	10

[a] This mobile phase was prepared at pHs: 3, 4.5, 6.5, the others were only prepared at pH 3.

The analysis was carried out with samples of extra virgin olive oil from different areas of Spain, which were subjected to MLC. To this end 50 μl of oil solutions (65% m/m) diluted with 2-propanol were injected directly. The olive oil samples analyzed were: Arboleda (Denomination Origin (D.O.) Bajo Aragón, Aragón, Spain), Reales Almazaras (D.O. Bajo Aragón, Aragón, Spain), Alcober (D.O. Bajo Aragón, Aragón, Spain), La Chinata (Plasencia, Cáceres, Spain), N[a] S[a] de Guadalupe (D.O. Baena, Andalucía, Spain), Valle de las Flores (D.O. Baena, Andalucfa, Spain) and Germán Baena (D.O. Baena, Andalucía, Spain). Optimisation of the separation of the phenolic compounds and phospholipids in the oil, and the recovery studies were carried out with the German Baena sample. To this end, solutions (containing 20% of olive oil diluted with 2-propanol), which were spiked with 100 μg/g of tyrosol, caffeic acid, *p*-coumaric acid, oleuropeina, phosphatidylethanolamine and phosphatidylcholine were used.

3. Results and discussion

Prior to optimisation of the separation of the different phenolic compounds by MLC, the corresponding chromatogram using reverse-phase-HPLC was obtained in order to compare the elution order of analytes, retention times, analysis times etc., with the results obtained by MLC. Using the gradient separation proposed by Mateos et al. (2001), the mobile phase was modified using (2%) acetic acid in water as eluent A and (2%) acetic acid in methanol as eluent B. The programme used can be seen in Table 2 and the chromatogram obtained in Fig. 1.

As previously pointed out, phospholipids have not been determined by reverse-phase HPLC but they can be determined by MLC, as it will be seen later. The use of a surfactant (SDS) which is adsorbed onto the surface of the stationary phase creates a hydrophilic film with a negative charge due to the presence of the sulphate group of the SDS, which enables the phospholipids to be separated in a C-18 column according to their polar heads.

Table 2
Final program for the separation of phenolic compounds by reverse-phase HPLC

Time (min)	Concentration of eluent B[a] (%)
0–10	0–30
10–11	40
11–30	40
30–40	40–60
40–50	60
50–57	100

[a] Eluent A: (2% m/v) acetic acid in water, eluent B: (2% m/v) acetic acid in methanol.

3.1. Optimisation of the mobile phase in separation by MLC

The parameters optimised for the separation by MLC were: surfactant (SDS) concentration and type of organic modifier and its concentration. Optimisation of these parameters was based on the criteria of greater selectivity, greater separation efficiency and shorter analysis time.

3.2. Phenolic compounds

The surfactant concentration and organic modifier concentration affect the elution strength in the mobile phase. An increase in surfactant concentration may increase the elution strength of the analytes. But if the surfactant concentration is sufficiently high, the layer produced on the

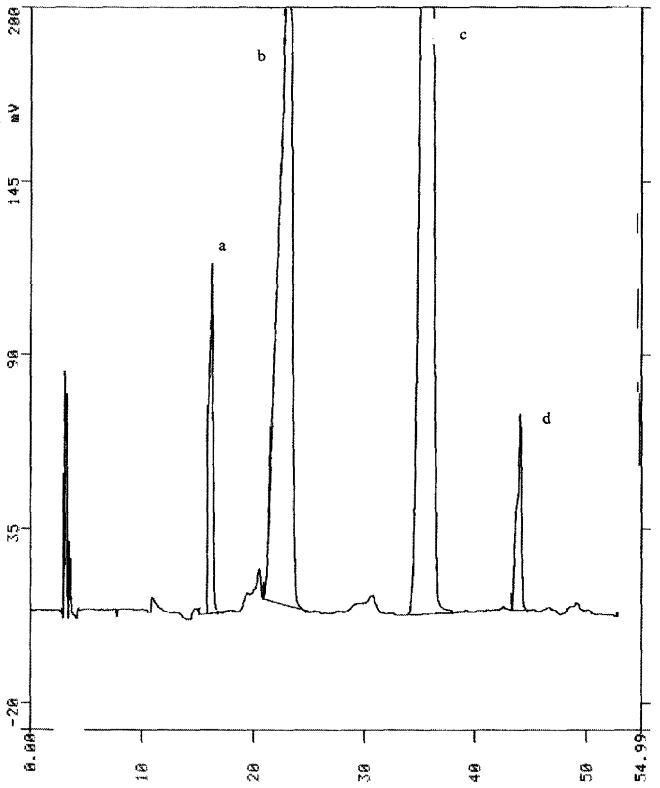

Fig. 1. Chromatogram obtained by reverse-phase HPLC for the standard solution:elution in gradient with methanol:water, $\lambda = 280$ nm: (a) tyrosol (16 min), (b) caffeic acid (22 min), (c) *p*-coumaric acid (36 min) and (d) oleuropeina (44 min).

stationary phase is so dense that it reduces efficiency due to the decrease in the mass transfer rate from the stationary phase. On the other hand, an increase in the organic solvent concentration always improves efficiency because it reduces the capacity factor of the analytes due to the elimination of the monomers in the stationary phase. In order to reach a compromise between these two interactive factors, a simultaneous optimisation strategy based on an iterative regression process (Rukhadze, Bezarashvili, Sebiskveradze, & Meyer, 1998; Strasters, Breyer, Rodgers, & Khaledi, 1990) or the use of Michrom software (Torres-Lapasió, García-Alvarez-Coque, & Baeza-Baeza, 1997) should be used.

In our case, the optimisation was developed using a standard solution containing tyrosol (125 mg/kg), caffeic acid (150 mg/kg), *p*-coumaric acid (125 mg/kg) and oleuropeina (100 mg/kg) in 2-propanol. The SDS concentration (0.03, 0.05 and 0.1 M) was varied using an initial pH 3, a flow rate of 1 ml min^{-1} and a constant initial 5% concentration of 2-propanol. When the SDS concentration was increased the retention times decreased owing to a larger number of micelles in the mobile phase but resolution of the four phenolic compounds was not achieved. Only caffeic acid and *p*-coumaric acid were resolved with 0.05 and 0.1 M in SDS. Tyrosol eluted with the solvent front and oleouropeina co-eluted with caffeic acid. Tyrosol was identified with 0.03 M SDS and 5% 2-propanol but not oleuropeina.

A reduction of the propanol concentration decreased the elution strength and increased the retention times, moving the analytes away from the solvent front. The influence of the SDS concentration (0.03, 0.05 and 0.07 M) was studied again with a 2.5% 2-propanol concentration. With 0.03 M of SDS only caffeic acid and *p*-coumaric acid were resolved with retention times of 13 and 18 min, respectively. With 0.05 M of SDS caffeic acid, *p*-coumaric acid and oleuropeina were resolved but tyrosol co-eluted with caffeic acid. All four compounds were resolved with 0.07 M of SDS and 2.5% of 2-propanol. The chromatogram obtained by MLC for the standard can be seen in Fig. 2. The elution order was tyrosol (6.7 min), caffeic acid (7.81 min), oleuropeina (8.30 min) and *p*-coumaric acid (9.70 min). A change in the elution order was observed if compared with reverse-phase as oleuropeina eluted last (44 min) probably because the interaction with the stationary phase in MLC depends on the electrostatic interaction with the charged SDS monomers rather than the polarity of the analytes.

3.3. Phospholipids

After optimisation of the mobile phase for the phenolic compounds, phosphatidylcholine and phosphatidylethanolamine (the most abundant phospholipids in olive oil) were identified and separated by introducing a standard solution containing the two compounds at a concentration of 100 mg/kg in 2-propanol. A wavelength of 210 nm was used for their detection (also employed for detection of

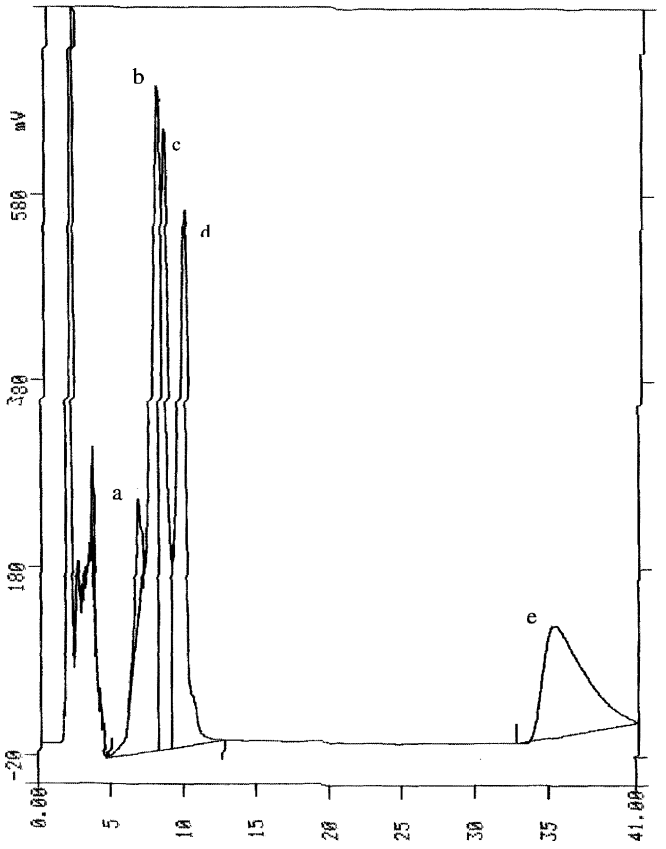

Fig. 2. Chromatogram obtained by MLC for the standard solution: mobile phase (0.07 M) SDS + (2.5%) propanol at pH = 3, flow rate: 0.8 ml min^{-1}, $\lambda = 210$ nm: (a) tyrosol (6.70 min), (b) caffeic acid (7.81 min), (c) oleuropeina (8.30 min), (d) *p*-coumaric acid (9.70) and (e) phosphatidylethanolamine (36 min).

the phenolic compounds) and there were no base line problems. As can be seen in Fig. 2, phosphatidylethanolamine eluted at 35.5 min and phosphatidylcholine at 57. min Taking the structure of these compounds into account, the high capacity factors are due to the interaction of the amine group with the SDS monomers adsorbed onto the stationary phase, and with the free silanol groups and the fatty acid chains, with the C-18 apolar stationary phase.

In order to reduce the retention times for these compounds an attempt was made to modify the mobile phase without affecting the separation of the phenolic compounds. Increasing the 2-propanol concentration from 2.5% to 5% did not reduce the elution time. An increase in pH (to deprotonize the amine group and charge the phosphate group negatively) to pH 6.5 (a pH of 2.5–7.5 is the most suitable for the column) did not change the retention times but did affect the resolution of the phenolic compounds. An organic modifier with greater elution strength was also used (1-butanol instead of 2-propanol). However, no significant changes were observed with a mobile phase composed of 0.07 M of SDS and 2.5% of 1-butanol as the retention time of phosphatidylethanolamine was only reduced from 35.5 to 32 min. Increasing the concentration of 1-butanol to 5% reduced the retention time of

phosphatidylethanolamine to 29 min Notwithstanding, the use of 1-butanol as an organic modifier may have a negative effect on samples like olive oil as 1-butanol is more likely to distort the micelles and eliminate the SDS monomers which protect the stationary phase. This may be a drawback for direct injection of the olive oil into the column. As the decrease in the retention times was not particularly significant, 0.07 M SDS + 2.5% 2-propanol at pH 3 was finally used as the mobile phase.

3.4. Direct injection of the sample into the column

As our main objective was to carry out the separation of the compounds studied without sample pre-treatment, and with direct injection of the sample into the C-18 column, the effect of injecting repeatedly increasing amounts of samples was studied. To this end, 50 μl of the (20%) German Baena olive oil sample in 2-propanol, spiked with 100 μg/g of tyrosol, caffeic acid, *p*-coumaric acid, oleuropeina, phosphatidylethanolamine and phosphatidylcholine were injected 7 times consecutively into the column with no increase of pressure in the column and the corresponding chromatograms were obtained. Unspiked samples with an olive oil content ranging from 30% to 65% were then gradually injected with no increase in column pressure. A slight increase in pressure was only observed after several consecutive injections of 50 μl of the olive oil sample with a 65% content and the increase was reversible and corrected itself in a few minutes.

The retention times and efficiency obtained for the spiked German Baena (20%) virgin olive oil sample and for the standard containing the analytes in 2-propanol can be seen in Table 3. It can be observed that there are hardly any differences as far as retention times are concerned. Therefore, it can be deduced that the presence of olive oil has no effect on the equilibrium of the analyte distribution in the stationary phase, the micelles and the mobile phase. This was also indicated by the efficiency, although *p*-coumaric acid and oleuropeina have longer tails when oil is not present, which deteriorates their efficiency. The mobile phase used, therefore, appeared suitable for direct oil sample injection.

If the retention times obtained by MLC (Table 3) are compared with those obtained by reverse-phase HPLC (Fig. 1) for the standard solution in 2-propanol, it can be seen that the retention times for the phenolic compounds are much lower when MLC was used than with reverse-phase HPLC (never under 20 min in other works, Carelli et al., 1997; Nomilos et al., 2002 and Singleton et al., 1995, using reverse-phase HPLC), although resolution and efficiency are better in reverse-phase HPLC. The main advantage of MLC is the possibility of injecting the olive oil sample directly without pretreatment. Furthermore, phenolic compounds and phospholipids can be separated in the same chromatogram (impossible by reverse-phase HPLC). Although the retention times for phospholipids by MLC are long, they are not much worse than those obtained by Nomilos et al. (2002) and Antonopoulou and Karantonis (2002) (16 min for phosphatidylcholine and 45 for phosphatidylethanolamine).

The chromatogram obtained for the direct injection of 50 μl of the German Baena (65%) virgin olive oil sample in 2-propanol can be seen in Fig. 3. The peaks correspond to: (a) tyrosol (6.4 min), (b) caffeic acid (7.5 min), (c) *p*-coumaric acid (9.4 min) and (i) phosphatidylethanolamine

Table 3
Values of retention times (min) and efficiency (N)[a] for: 1. (20%) Spiked olive oil sample in 2-propanol and 2. Standard solution in 2-propanol

	t (min)		Efficiency (N)	
	1	2	1	2
Tyrosol	6.7	6.7	233	256
Caffeic acid	8.0	7.8	1468	1585
Oleuropeina	8.3	8.3	1136	746
p-Coumaric acid	10.5	9.7	100	52
Phosphatidylethanolamine	35.2	35.2	163	210

[a] Efficiencies calculated by application of the Foley–Dorsey equation.

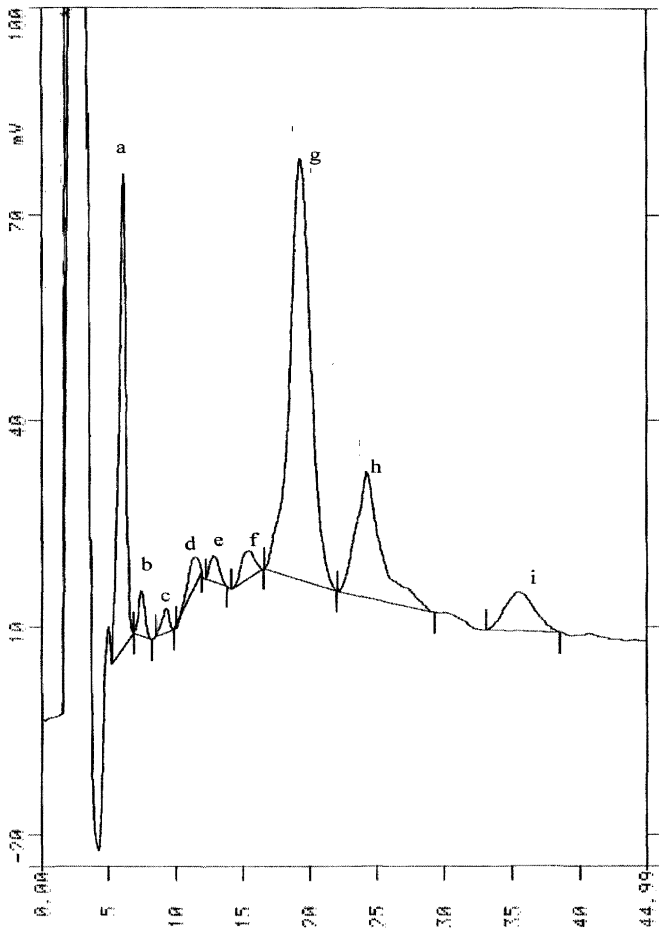

Fig. 3. Chromatogram obtained by MLC for the German Baena extra virgin olive oil sample (65% m/m) dissolved in 2-propanol: mobile phase (0.07 M) SDS + (2.5%) propanol at pH 3, flow rate: 0.8 ml min^{-1}, $\lambda = 210$ nm: (a) tyrosol (6.4 min), (b) caffeic acid (7.5 min), (c) *p*-coumaric acid (9.4 min), (i) phosphatidylethanolamine (35.5 min), (d–g): unknown compounds.

(35.5 min). Peaks d, f, g and h do not correspond to any of the compounds studied in this paper. No peak was observed for oleuropeina in the virgin olive oil (no peak was also observed for the other olive oils analysed subsequently).

3.5. Figures of merit

Calibration parameters and figures of merit, obtained by injecting standard solutions in 2-propanol are given in Table 4. The limits of detection (LODs) were calculated as three-fold the standard deviation of the baseline (12 measurements were taken), divided by the slope of the calibration curves obtained from peak height. Repeatability was evaluated by performing replicated injections ($n = 4$) of the spiked sample to include the matrix effect: German Baena (65%) virgin olive oil in 2-propanol. Values for oleuropeina are not given as it was not found in the oils studied. The detection limits are in the 0.052–0.16 μg/g range and are similar to those found by Pirisi et al. (1997) using reverse-phase chromatography. The repeatability values range from 1% to 8.6% for 12 the phenolic compounds. A high degree of imprecision is observed (21.9%) for phosphatidylethanolamine which has a much higher capacity factor than the phenols. Linear curves were obtained in the range of concentrations studied (up to 150 μg/g) and better linear regression coefficients were obtained for peak heights than peak areas. The recovery values for the Germán Baena virgin olive oil sample were calculated introducing a spiked sample. The values obtained (which can be considered satisfactory) and the content of the different compounds analyzed in the different olive oil samples from different areas of Spain are given in Table 4. The concentrations are in good agreement with those obtained for other samples using reverse-phase chromatography(Nergiz & Ünal, 1991; Pirisi et al., 1997; Tsimidou, Papadopoulos, & Boskou, 1992). As it can be seen, the caffeic acid concentration is in many cases, below the detection limit (Table 5).

It is noteworthy that, although not many samples were analyzed, the olive oils from the south of Spain (Nuestra Sra. De Guadalupe, Valle de las Flores and German Baena) have a higher content of tyrosol and *p*-coumaric acid than the oils from Bajo Aragón (Arboleda, Reales Almazaras and Alcober). In a previous paper published by Jimenez, Velarte, Gómez, and Castillo (2004), different types of Spanish virgin olive oil were classified according to their metallic content and were differentiated on the basis of their Al content (related to the type of green olive) and Mn and Ba (related to the type of soil). It was also observed that oils from the south of Spain had a higher metallic content than those from Bajo Aragón. It would, therefore, appear that the type of olive, climatic conditions, the type of soil and the area in the south of Spain favour the synthesis of a higher concentration of polar compounds (phenols), which also favours the dissolution of metals.

Table 4
Calibration parameters and analytical figures of merits obtained by injecting standard solutions in 2-propanol and measuring peak areas (normal typefaces) and heights (italics)

	B[a]	A[a]	r^2	LOD (μg/g)	QL (μg/g)	RSD (%)[b]
Tyrosol	1.6	−32.0	0.997	0.052	0.17	7.2
	3.6	*−84.6*	*0.999*			*6.5*
Caffeic acid	5.9	−193.0	0.992	0.02	0.07	8.4
	6.5	*−63.0*	*0.997*			*8.6*
p-Coumaric acid	3.7	−73.7	0.995	0.05	0.17	5.7
	4.6	*−63.0*	*0.999*			*1.0*
Phosphatidylethanolamine	4.3	−35.2	0.998	0.16	0.53	22.1
	1.6	*−9.6*	*0.997*			*21.9*

[a] $Y = A + Bx$.
[b] Repeatability, as relative standard deviation for spiked olive oil sample (20%) in 2-propanol ($n = 4$).

Table 5
Quantitative results for analysis of different Spanish virgin olive oil samples

	Tyrosol (μg/g)	Caffeic acid (μg/g)	*p*-Coumaric acid(μg/g)
Arboleda	3.39 ± 0.22	1.45 ± 0.12	0.80 ± 0.008
Reales Almazaras	3.14 ± 0.20	0.15 ± 0.01	0.95 ± 0.009
Alcober	3.39 ± 0.22	<LD	0.86 ± 0.009
La Chinata	4.7 ± 0.30	<LD	2.61 ± 0.03
N[a] S[a] de Guadalupe	8.87 ± 0.58	<LD	3.65 ± 0.04
Valle de las flores	8.68 ± 0.57	0.49 ± 0.04	1.30 ± 0.013
Germán Baena	9.6 ± 0.62 (112)[A]	1.5 ± 0.01 (107)[A]	2.30 ± 0.02 (113)[A]

[A] Values in parenthesis: recoveries (%).

4. Conclusions

A procedure was developed for the separation and determination of tyrosol, caffeic acid, *p*-coumaric acid, oleuropeina (phenolic compounds), phosphatidylethanolamine and phosphatidylcholine (phospholipids) in virgin olive oil samples by MLC. This method permits direct sample injection without pretreatment and so the procedure is relatively fast. If only phenolic compounds are determined the chromatogram is obtained in 10 min. If compared with determination of these compounds by reverse-phase HPLC with previous solid-phase extraction, the method is much faster and contamination problems are avoided. The LODs, reproducibility values and recovery values are quite satisfactory (especially for the phenolic compounds) and the procedure could therefore, be used for the routine determination of these compounds in virgin olive oil.

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