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Determination of free fatty acids in olive oils by UPHLC–MS

Saikh M. Wabaidur[a,*], Ahmad AlAmmari[a], Ahmad Aqel[a,*], Saad A. AL-Tamrah[b], Zeid Abdullah Alothman[a,b], A.Y. Badjah Hadj Ahmed[a]

[a] Advanced Materials Research Chair, Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[b] Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

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ABSTRACT

A simple, fast, highly efficient and direct method using ultra-performance liquid chromatography coupled to mass spectrometry has been established for the simultaneous separation, identification and quantitation of a few saturated and unsaturated fatty acids in olive oils from various countries. No sample pretreatment techniques were employed such as extraction or derivatization for the analysis of target acids from oil samples, as the oil samples were just diluted, filtered and then directly injected to the instrument. The chromatographic separations of all target fatty acids were achieved on a Hypersil Gold C_{18} column of particle size 1.9 μm, 50 × 2.1 mm I.D, while the gradient elution using a binary mobile phase mixture of acetonitrile and water at a flow rate of 1.5 ml/min was adopted for achieving optimum separations. The identification and quantitation of target compounds was accomplished using selected ion reaction monitoring mode. The recoveries of the fatty acids were obtained higher than 89% with good validation parameters; linearity ($r^2 > 0.992$), detection limit between 0.09 and 0.24 μg/ml, run to run and day to day precisions with percent relative standard deviation lower than 2.4% at both low (1 μg/ml) and medium (10 μg/ml) concentration levels. The total content of fatty acids in each individual oils was found in the range of 472.63–7751.20 μg/ml of olive oil, while oleic acid was found to be the major fatty acid among all analyzed oils with the amount 3785.94 μg/ml (maximum) in Syrian olive oil. The obtained validation parameters confirm that the proposed analytical method is rapid, sensitive, reproducible and simple and it could be applied for the successful evaluation of fatty acids in various oils and other matrices. All the fatty acids were efficiently eluted in a time of less than 8 min with well resolved peaks by employing the proposed method.

1. Introduction

Application of edible oil in food items has become a part of the cuisine in every nation around the world. The flavor and taste of the food product mostly depends on the types of oil which was used during the food preparations [1]. According to the European consumers, olive oil is most demanded among the various edible oils as it has a market share of around 20% [2]. Oils are commonly found in nature as triglyceride, which is an ester of glycerol and fatty acids (FAs). Three hydroxy groups of glycerol are combined with either saturated or unsaturated FAs through esterification to form the triglyceride. Thus, all the edible oils are rich of both saturated and unsaturated FAs [1]. The common FAs that are usually present in the oils are tridecanoic acid ($C_{13:0}$), myristic acid ($C_{14:0}$), pentadecanoic acid ($C_{15:0}$), palmitic acid ($C_{16:0}$), margaric acid ($C_{17:0}$), stearic acid ($C_{18:0}$) (saturated acids), oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) (unsaturated acids) [3,4]. But the composition of the FAs varies in the oils and they contain high proportion of unsaturated FAs compared to saturated FAs [1].

Both saturated and unsaturated FAs are play vital role with many biological activities in food, oil and living organism [5,6] and the quality indices of the oils during the production, storage, and trading are mainly determined on the basis of their FAs contents [7,8]. In addition, the analysis of FAs composition has been used to optimize the oil refining, authenticity of the oil and control the degradation of oil under varied circumstances [9]. Sometimes, FAs analysis has also been used to detect the adulterations of high quality olive oil with other cheap oils [1]. Hence, the level of FAs composition in oil is directly correlated to the quality and as well as the authenticity of the oils. Therefore, considering the significant role of FAs in quality control of oils, it is obviously demanded to develop a simple, rapid and trustworthy analytical technique to identify and quantitate the FAs compositions in oils and as well as some other related matrices.

* Corresponding authors.
E-mail addresses: tarabai22@yahoo.com.sg (S.M. Wabaidur), aifseisi@ksu.edu.sa (A. Aqel).

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Although, it is relatively difficult and quite challenging to develop the separation and determination method of FAs as they present in relatively low concentration in the highly complex matrix [10]. There are many analytical techniques can be found in the literature for the determination of FAs. The methods include either the derivatization or esterification of FAs. For example, the European Union has established a titration based official method for FAs determination in olive oil but the technique is not appropriate for process control purposes, since it is time-consuming, laborious and requires large amounts of solvents [11]. To overcome such problems of the official method, several spectroscopic methods including Fourier transform infrared (FTIR) spectrometry has been proposed [12–15]. These methods were able to provide probable alternatives to the official method but could not avoid sample treatment or reaction. Only attenuated total reflection (ATR)–FTIR was directly measure the FAs composition in the oil but it needs different rinsing solutions to clean the surface of the ATR element before each sample measurements [14].

Gas chromatography (GC) and GC with mass spectrometry (GC–MS) have been reported for indirect analysis of FAs as methyl esters derivative with improved resolution in the last decades [16–21]. But the sample derivatizations for trace amount analysis of FAs in edible oils are tedious and time-consuming, since direct determination is not possible as FAs are non-polar and not volatile [22]. GC and GC–MS methods also face big problems when apply to real sample analysis due to incomplete derivatization of FAs [22,23]. Moreover, by-products formations during esterification of fatty acids, thermal degradation and risks re-arrangements of double-bond have been the major problems for these methods [22,24]. Liquid chromatography (LC) techniques with various detection methods have also been attempted for FAs analyses [25–27]. However, due to the weak absorption and fluorescent properties of FAs complications still exist with these methods [28]. Thus, pre- or post-column derivatization of FAs such as esterification or incorporation of appropriate and strong chromophore or fluorophore is necessary with the aim to achieve efficient separation and increase the detection sensitivity of HPLC. Many HPLC methods coupled with various detections including fluorescence, Photo diode array, ultraviolet–visible adsorption and evaporative light scattering for analysis of FAs are described in the previous scientific studies [29–31]. During the derivatization of FAs few parameters play very crucial role such as, amount of derivatizing reagents, reaction temperature and time taken by the reaction to avoid formation of any by-product and achieve high reaction efficiency [28]. In addition, HPLC technique needed comparatively longer analysis time and consume enormous quantities of solvent. Hence, it is of high demand to develop a simple, fast and efficient method for the analysis of FAs.

Hyphenated ultra-performance liquid chromatography coupled with mass spectrometry (UHPLC–MS) has been a promising tool to overcome the aforementioned limitations such as sample pretreatment for the analysis of FAs. Many important progresses have been achieved in recent years with this technique, especially dealing with real samples and their direct injection onto the column which demonstrates that UPHLC–MS has great application to check the quality and detect adulteration of oil to circumvent various health risks [1]. Therefore, in the current paper, an UPHLC–MS method has been discussed for the novel, rapid, reliable, direct detection and accurate quantification of FAs compositions of olive oil. Selected ion recording (SIR) acquisition was applied for the identification and confirmation of molecular ion peaks of the pure target compounds and their respective peak areas were used for the quantitative analyses of them. The proposed method has shown many advantages compared to the reported method which involves sample pretreatment and able to accurately determine the individual FAs in olive oils without any interference as traditional derivatization reagents were not required. Hence, it will be useful to assess the FAs profiling of various oils. All the analyzed FAs were eluted in <8 min with successful resolution of the peaks.

2. Materials and methods

2.1. Standards and reagents

Tridecanoic acid (C13:0, P99%), myristic acid (C14:0, P99%), pentadecanoic acid (C15:0, P99%), palmitic acid (C16:0, P99%), margaric acid (C17:0, P99%), linoleic acid (C18:2, P99%), oleic acid (C18:1, P99%) and stearic acid (C18:0, P99%) standards were obtained from Sigma Aldrich (St. Louis, MO, USA). HPLC grade *n*-hexane, methanol, formic acid, isopropanol and acetonitrile were bought from BDH Laboratory Supplies (BDH Chemicals Ltd., Poole, UK). UPLC-grade water was acquired from Milli-Q water purification system (Millipore, Bedford, MA, USA). All other solvents were used of analytical grade. Stock solutions were prepared in isopropanol at a concentration of 1000 μg/ml and were diluted with isopropanol to get a series of desired concentration of 0.1, 0.3, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 150.0 and 200 μg/ml. All stock solutions were kept at 4 °C. Mixture of standard solution was prepared by mixing the specific fatty acid stock solutions and then diluted with isopropanol to get the desired concentration level.

2.2. Oil materials

A total of 8 olive oil samples of different brands were purchased from local supermarkets. At least five samples of each brand were randomly selected and mixed together and 50 μL aliquot from each mixture were separately dissolved in isopropanol to make a final total volume of 1 mL. Then the vial was vigorously shaken for 1 min and filtered with 0.22 μm Polytetrafluoroethylene (PTFE) filter. Finally, 4 μl aliquot of the filtrate was directly injected into the UPLC–MS instrument for analysis without further treatment.

2.3. Instrumentation

2.3.1. Ultra performance liquid chromatography (UPLC)

UPLC analysis of fatty acids were performed on an Acquity UPLC system (Waters Corp., Milford, MA, USA), fitted with a binary solvent manager, a sample manager and column heater.

The liquid chromatographic experiments of all analyzed FAs were achieved using a Hypersil Gold C_{18} column (50 × 2.1 mm i.d., particle size: 1.9 μm) (Thermo Fisher Scientific, Waltham, MA USA). The Acquity UPLC system (Waters®, Manchester, UK) equipped with an Acquity UPLC binary solvent manager and sample manager and a column heater. The column temperature was kept at 60 °C and the sample manager was maintained at room temperature. Sample and mobile phase filtration was carried out using a Welch Duo-Seal rotary pump (Model No.1400, USA). Grant-bio PV-1 vortex mixer (Cambridge, England) was used for mixing the solutions. The sample injection volume was 4 μl. To remove any retained compounds from the column it was washed with 100% acetonitrile after each run.

2.3.2. Mass spectrometry (MS) measurements

A Quattro Premier triple-quadrupole mass spectrometer equipped with electrospray ionization (ESI) source of Micromass Company Inc. (Manchester, UK) was used for mass spectrometry measurements. The ESI source was used for ionization of target compounds. A SOGEVAC SV40 BI Oerlikon rotary pump (Paris, France) was produced the significant vacuum in the mass spectrometer. All the mass spectrometry measurements have been performed using electrospray ionization with negative mode

Table 1
Detailed gradient flow profile for UPHLC system for the analysis of fatty acids using Hypersil Gold C_{18} column and the column temperature was kept constant at 60 °C.

Time (min)	ACN:Milli Q water (%)	Flow (ml/min)	Curve
Initial	42:58	1.5	–
3	42:58	1.5	4
5	57:43	1.5	4
8	100:00	1.5	–

(ESI$^-$). Monitoring conditions were optimized for achieving highest peak intensity. The specific cone voltage was optimized for the formation of parent ions. High-purity nitrogen gas created by a nitrogen generator of Peak Scientific NM30LA (Inchinann, UK) was supplied to the ion source for nebulizing purpose. All experimental data collection was carried out by MassLynx V4.1 software (Micromass, Manchester, Lancashire, UK).

3. Results and discussion

3.1. Optimization of UPHLC conditions

The initial separations of all the physiologically important FAs were carried out using a mixture of standard solution of six saturated and two unsaturated FAs. The liquid chromatographic parameters including column, column temperature, compositions of mobile phase and the flow rate of the mobile phase were optimized to acquire the best resolution of the peaks and to minimize the peak tailing. The reversed phase columns of various lengths such as BEH C_{18} (50, 100 or 150 mm) and Hypersil Gold C_{18} (50, 100 or 150 mm) were tested. The best separations with low run time were achieved with Hypersil Gold C_{18} column (50 × 2.1 mm i.d., 1.7 μm particle sizes). Similarly, mobile phases of various compositions of water, methanol, acetonitrile and aqueous formic acid 0.1% (V/V) were predicted at various flow rates ranging from 0.05–2 ml/min using both gradient and isocratic elution modes. Flow rate is chromatographic analysis is one of the important aspect for achieving the best retention times and effective separation to avoid wastage of time, excess manpower and the extra chemical used in the mobile phases. Therefore, to acquire the best resolution of the peaks and to minimize the peak tailing in the shortest run time, 0.5, 1.0, 1.5, and 2.0 ml min^{-1} flow rates were tested and the relation between the flow rate and the mean area of the components were established. During the optimization, it has been observed that the peak intensity and resolution was better while using 1.5 ml flow rate. Although, lower better retention time was achieved with 2.0 ml flow rate, but a sharp decrease in the peak area and high column backpressure were observed. Thus, 1.5 ml was selected for analysis purpose.

To investigate the effect of temperature on the separation, the column temperature was optimized from 25 to 80 °C. The optimum separation was achieved using gradient elution with a binary mobile phase mixture of water (A) and acetonitrile (B) according to the parameters given in Table 1. The shortest analysis time of 8.0 min was achieved using the flow rate 1.5 ml/min, while the total analysis time was 12 min. From the Table 1 it is obvious that, initially the composition of A was 58% and flow rate 1.5 ml/min for 3 min. Then the composition of A was decreased to 43% between 3 and 5 min using gradient curve 4 and finally the composition of A is decreased to 0 between 5 and 8 min. The column was heated at a fixed temperature of 60 °C to lower the back pressure of the column. During the chromatographic analysis, triglycerols can strongly be retained on C_{18} column and can produce a background and noise. Therefore, to avoid such background signal, the column was washed with 100% acetonitrile after each run. In addition, the efficiency testing of the column was evaluated based on injected sample volume of 1, 2, 3 and 4 μl. It was noted that an increase in sample volume does not cause a significant broadening or changes in the number of theoretical plate. Therefore, we choose 4 μl as optimum injection volume because our home-made full sample loop volume was 4uL, which provides good repeatability for the results during the quantitative analysis.

3.2. Mass spectrometry (MS) parameters optimizations

The optimization process was carried out to obtain the best peak intensity of the target analyte. Direct infusion of each individual target analyte (5 μg/ml) was done to the ion source of the MS detector to obtain the highest molecular ions peak. Both positive and negative electrospray ionization (ESI) modes were tested, while better and highly abundant analyte signals were detected in negative ionization mode. Thus, the negative electrospray ionization (ESI$^-$) mode was chosen for further experiment. The MS parameters such as, cone voltage, capillary voltage, desolvation temperature, source temperature and desolvation gas flow were studied in the range of 10–100 V, 2.0–4.5 kV, 200–450 °C, 90–150 °C and 500–800 l/h, respectively. The optimized MS conditions were found to be as follows: capillary voltage 3.5 kV, cone voltage 40 V, Extractor 2 V, RF lens 0.1 V, source temperature 120 °C, desolvation temperature 300 °C, desolvation and cone gas flows were 600 and 60 l/h, respectively. The identification and quantitation was achieved using selected ion reaction (SIR) mode for each fatty acids. The SIR data acquisition parameters for each individual acids including abbreviation, retention times, molecular formula, molecular weight, cone voltage and precursor ion ([M−H]$^-$) are listed in Table 2. The UPLC–MS chromatogram obtained using the optimal experimental conditions for the mixture of eight analyzed fatty acids is shown in Fig. 1. It is obvious from the figure that the proposed analytical technique has addressed so many issues including sharp and symmetric peak, and good resolution without any peak tailing, although, the effective separations of each components are not necessary in MS detection but it brings further improvement of selectivity and sensitivity to the methodology [32].

3.3. Validation of the proposed UPHLC–MS procedure

3.3.1. Calibration and linearity

The quantitative analysis of all FAs was accomplished by comparison of peak area with linear calibration curve of the analytes. Construction of linear regression curves was performed by prepared a series of calibration standards of each FA in isopropanol of concentration 0.1, 0.3, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 150.0 and 200 μg/ml. Under the optimum UPLC–MS conditions the linear relationship of the proposed method was achieved by plotting the graph between analyte peak area and concentration, while the equation of linear regression for each analyte was established using least squares method. The linear response of the calibration curve was found over the concentration range of 0.5–50 μg/ml for C17:0, 1– 50 μg/ml for C16:0 and 1–100 μg/ml for rest of the FAs, while the correlation coefficient (r^2) values was found to be >0.992 for each analyte. The calibration data and regression equations are presented in Table 3.

3.3.2. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ values were calculated considering the lowest quantity of target analyte that provides a chromatogram peak with a signal-to-noise (S/N) ratio 3:1 and 10:1 as the background signal noise, respectively. The S/N values were calculated by analyzing three replicates of spiked FAs solution at the concentration levels of 0.05, 0.10, 0.50, 0.75 and 1.0 μg/ml [33]. The obtained LOD and

Table 2
The abbreviations, optimized SIR parameters, retention times (t_R) and peak resolutions (R_s) for each individual FAs (dwell time = 0.025 s).

Fatty acids	A.F.[a]	t_R (min)	R_s (N[c]/m)	Mol[b] formula	Mol[b] weight	Precursor Ion $[M-H]^-$, *m/z*	Cone Voltage (V)
Tridecanoic acid	C13:0	1.68	– (37840)	$CH_3(CH_2)_{11}COOH$	214.35	213.35	38
Myristic acid	C14:0	2.61	5.36 (58460)	$CH_3(CH_2)_{12}COOH$	228.38	227.35	40
Pentadecanoic acid	C15:0	4.06	6.55 (83680)	$CH_3(CH_2)_{13}COOH$	242.40	241.35	42
Linoleic acid	C18:2	4.43	1.43 (85920)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	280.45	279.42	42
Palmitic acid	C16:0	5.80	5.93 (288640)	$CH_3(CH_2)_{14}COOH$	256.43	255.42	42
Oleic acid	C18:1	6.28	2.63 (417780)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	282.47	281.42	44
Margaric acid	C17:0	6.72	2.94 (605420)	$CH_3(CH_2)_{15}COOH$	270.45	269.42	46
Stearic acid	C18:0	7.63	5.31 (618700)	$CH_3(CH_2)_{16}COOH$	284.48	283.42	48

[a] A.F.: Abbreviated form.
[b] Mol: Molecular.
[c] N: Plate number.

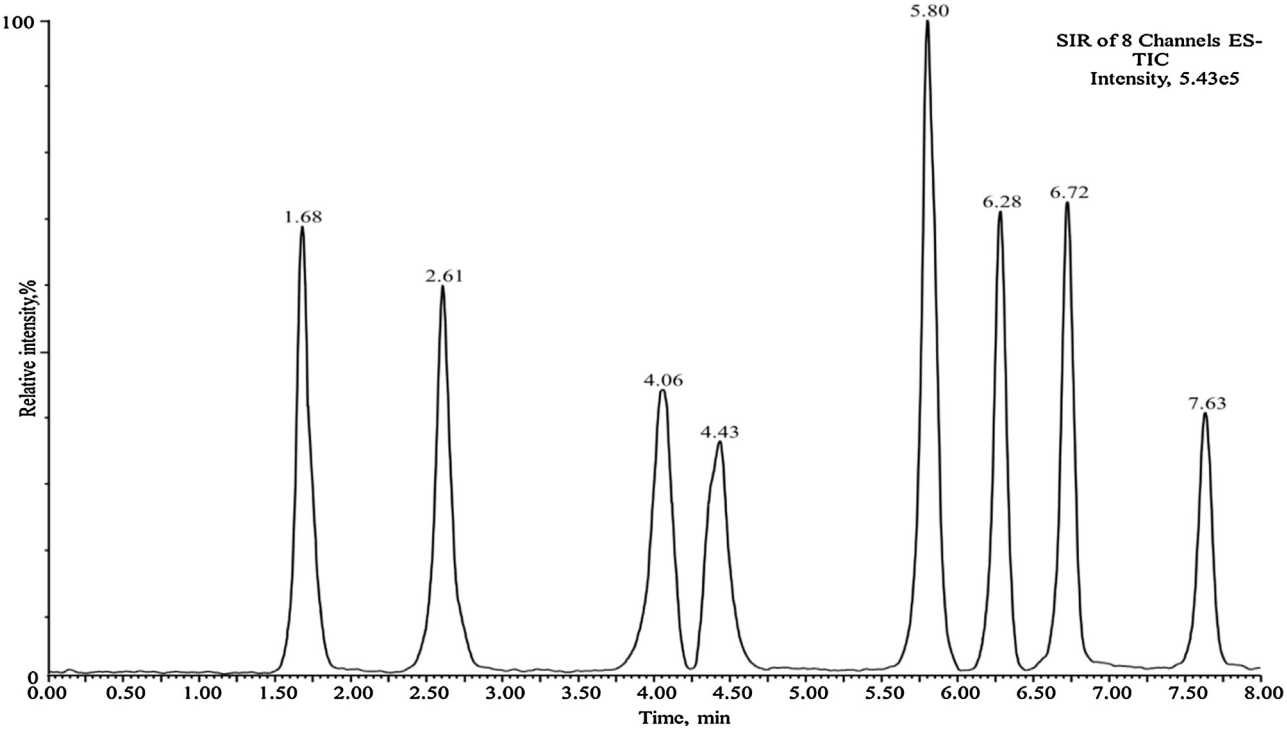

Fig. 1. The UPHLC–MS total ion chromatogram of standard fatty acid in mixture of concentration 5 μg/ml using Hypersil Gold column (1.9 μm, 50 × 2.1 mm i.d.). Peak identifications: 1.68 min (C13:0, 213.35 *m/z*), 2.61 min (C14:0, 227.35 *m/z*), 4.06 min (C15:0, 241.35 *m/z*), 4.43 min (C18:2, 279.42 *m/z*), 5.80 min (C16:0, 255.42 *m/z*), 6.28 min (C18:1, 281.42 *m/z*), 6.72 min (C17:0, 269.42 *m/z*) and 7.63 min (C18:0, 283.42 *m/z*).

Table 3
The calibration parameters for the proposed method.

Fatty acids	Concentration range (μg/ml)	Linear Regression line	Correlation Coefficient (r^2)	LOD (μg/ml)	LOQ (μg/ml)	[a]RSD (%)
C13:0	1–100	y = 3845.6x + 21927	0.9929	0.16	0.51	1.6
C14:0	1–100	y = 4659.8x + 18407	0.9954	0.23	0.71	2.0
C15:0	1–100	y = 5024.8x + 14299	0.9947	0.23	0.70	1.8
C18:2	1–100	y = 5489.7x + 13707	0.9943	0.09	0.29	1.9
C16:0	1–50	y = 7102.1x + 27707	0.9940	0.24	0.71	2.1
C18:1	1–100	y = 4676.5x + 17084	0.9967	0.20	0.61	2.4
C17:0	0.5–50	y = 6878.1x + 1016.7	0.9989	0.13	0.40	2.2
C18:0	1–100	y = 3978.6x + 5876.3	0.9982	0.22	0.65	1.5

[a] RSD was calculated for 10 repeated analysis.

LOQ values for all FAs were in the range of 0.09–0.24 μg/ml and 0.29–0.71 μg/ml, respectively (Table 3).

3.3.3. Accuracy and precision

The intra-day and inter-day precisions of the proposed UPHLC–MS method were tested. Three replicates of the standard FAs mixture of concentration 1, 4, 7 and 10 μg/ml were run thrice in the same day for intra-day and twelve replicates during three consecutive days (three replicates per day) for inter-day precisions [34]. Intra-day and inter-day precisions in terms of RSD in both cases were achieved less than 2.4% and that is within the acceptable range. Also the precision with 2.4% RSD confirms the effective applications of the developed UPHLC–MS method for the routine analysis of saturated and unsaturated FAs in various oil samples. Furthermore, during the validation process, the slight changes of the optimized experimental parameters does not affects the results, so the method proved to be robust.

Table 4
FAs concentrations in the analyzed oil samples (n = 3) (μg/ml oil.) and their determined recovery rates (R, %).

Source country of olive oil	Fatty acids, μg/ml ± SD[a]								Total
	C13:0(R)	C14:0(R)	C15:0(R)	C18:2 (R)	C16:0 (R)	C18:1 (R)	C17:0 (R)	C18:0 (R)	
Saudi	nd (92)	nd (95)	nd (95)	995.64 ± 2.5 (95)	896.32 ± 2.3 (95)	3159.84 ± 1.6 (95)	5.07 ± 0.7 (96)	626.72 ± 2.3 (95)	5683.59 ± 2.6
Serian	nd (94)	nd (97)	nd (95)	1310.31 ±2.5 (92)	1383.11 ±2.3 (95)	3785.94 ± 3.6 (97)	310.67 ± 1.6 (95)	961.21 ± 2.5 (94)	7751.20 ± 3.2
Italian	nd (92)	nd (99)	nd (96)	171.39 ±2.6 (91)	153.38 ±2.3 (93)	1556.23 ±1.9 (89)	2.54 ± 0.5 (95)	82.86 ± 1.3 (94)	1966.40 ± 1.8
Tunisian	nd (95)	nd (92)	nd (91)	994.76 ± 2.5 (92)	772.28 ± 2.2 (93)	2455.41 ± 2.6 (98)	0.156 ± 0.6 (92)	274.63 ± 1.4 (92)	4497.24 ± 2.5
Turkish	nd (93)	nd (95)	nd (93)	876.23 ± 2.5 (93)	915.76 ± 2.3 (92)	3292.53 ± 3.5 (95)	4.67 ± 0.5 (92)	558.72 ± 1.3 (95)	5647.91 ± 2.6
UK	nd (96)	nd (97)	nd (94)	56.14 ± 1.5 (93)	104.62 ± 1.8 (93)	229.30 ± 1.6 (95)	6.94 ± 0.6 (97)	75.63 ± 0.8 (90)	472.63 ± 1.1
Spanish	nd (94)	nd (90)	nd (98)	84.51 ± 1.6 (92)	33.05 ± 1.1 (92)	1327.23 ± 1.9 (97)	5.76 ± 0.6 (97)	89.48 ± 0.9 (90)	1540.03 ± 1.6
Algerian	nd (96)	nd (94)	nd (93)	953.57 ± 2.1 (92)	710.94 ± 2.2 (93)	2605.58 ± 1.6 (94)	2.90 ± 0.6 (95)	201.79 ± 0.4 (92)	4474.78 ± 2.6

R = Recovery; nd-not detected; bd-below detection limit.
[a] Mean of three determinations ± standard deviation.

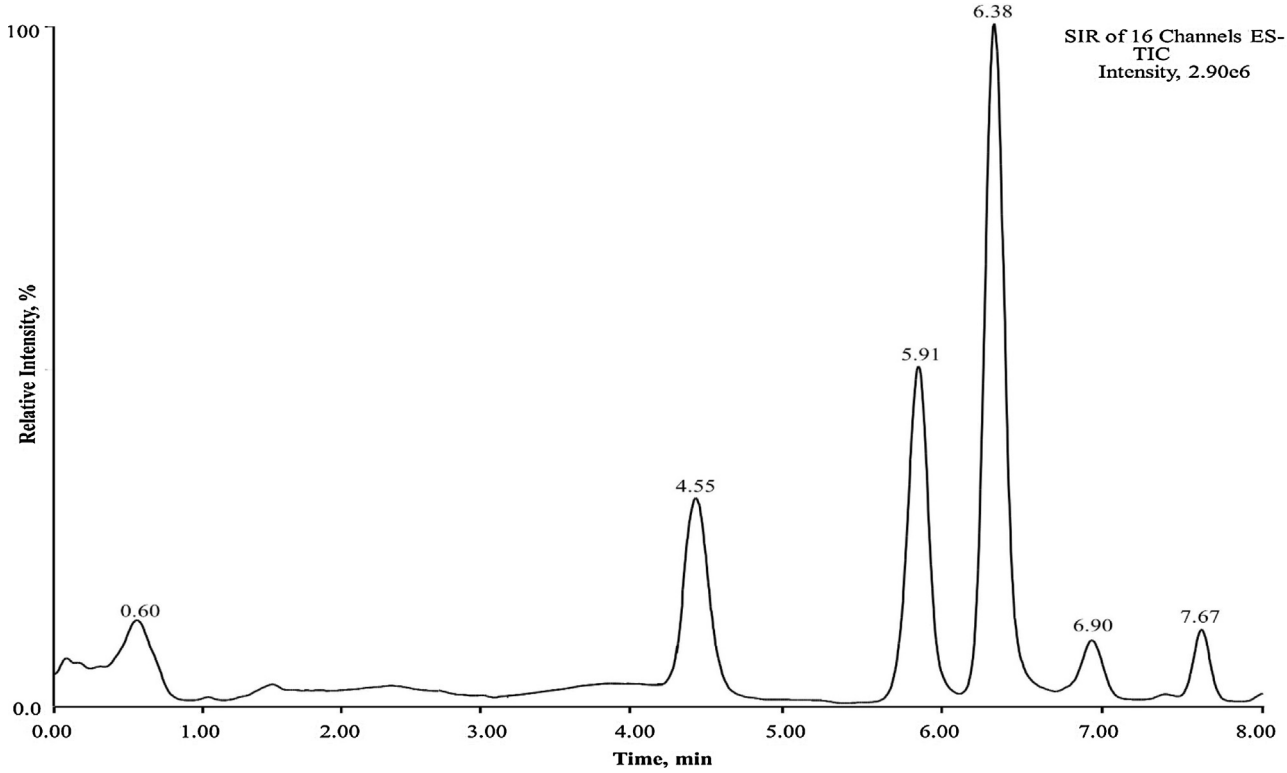

Fig. 2. UPHLC–MS chromatogram of Serian olive oil using optimum parameters.

The recovery test was carried out to check the applicability of the proposed UPHLC–MS technique. Three replicates of standard mixture at low-, medium-, and high-concentration level (within the calibration range) were added to each olive oil sample before filtration for recovery measurements. The average recoveries of the analytes were found in the range of 89–98% with RSD between 1.0–2.1% (Table 4).

3.3.4. Matrix effect

There are many experimental protocols can be found for evaluating the occurrence of ion suppression in LC technique. In our study matrix matched calibration standards technique was used to see the ion suppression effect. For this, calibration standards of free FAs were prepared in identical sample matrix to be analyzed (e.g. oil) by spiking a known concentration of standard analyte. The sample matrix was free of the FAs of interest. Both the Matrix free and matrix calibration of free FAs samples were analyzed and almost same peak areas or peak heights with less than 3% suppression were found. The smaller ion-suppression value was found since the extent of ion-suppression for Z-spray geometry of the ESI source is much lower than orthogonal spray and linear spray [37]. Also, the introduction of small quantity od mobile phase additives such as formic acid, decrease the suppression of ESI signal and provide better efficiency [38].

3.3.5. Carry-over study

Under the optimal chromatographic and MS conditions, the separation of the entire target FAs were achieved within a 8-min run time, but the analysis was allowed for 12 min. The addition of extra 4 min to the end of the run between samples using 100% ACN effectively washed the system thereby eliminating carry-over and allowed the column to be stabilize and ready for the next injection.

3.4. Application: analysis of FAs in olive oil

The simultaneous determinations of FAs in olive oil of eight different origins including Saudi, Seria, Italy, Tunisia, Turkey, UK, Spain and Algeria have been carried out by employing the pro-

posed method. All the oil samples were directly taken and diluted with isopropanol as described in "oil material" section and then 4 μl of it was injected into the UPHLC–MS system without derivatization or extraction. The peaks found in the chromatograms were then identified by the comparison of retention times and molecular ions spectra of standard mixture of FAs that recorded under the same analytical parameters [35,36]. For example, the UPHLC–MS chromatogram of Syrian olive oil is shown in Fig. 2. It is obvious from the figure that all the FAs are well resolved from each other and no noticeable matrix peaks were eluted at the retention time of the analyzed compounds that confirms no interferences were occurred from any other components [39]. The obtained results of the FAs composition of the analyzed oils have been shown in Table 4. The total amount of FAs in each individual oils was found in the range between 472.63–7751.20 μg/ml of oil, while unsaturated fatty acid (C18:1) was found to be the major fatty acid (229.30-3785.94 μg/ml of oil) in all analyzed oils, which are in agreements with the previously reported results [1,]. The maximum C18:1 acid contents of 3785.94 μg/ml of oil was found in Syrian olive oil and lowest level of this acid content (229.30 μg/ml of oil) was found in UK olive oil. The doubly unsaturated FAs, C18:2 was also present in large quantities (maximum 1310.31 μg/ml of oil in Syrian) in all types of olive oils except in UK oil (56.14 μg/ml of oil) (Table 4).

Among the saturated FAs, C16:0 and C18:0 were also obtained in large quantity in Saudi, Syrian, Tunisian, Turkish and Algerian olive oil, while they were present in comparatively lower amount in Italian, UK and Spanish olive oil. The FAs C17:0 was found in relatively lower amount in all analyzed oils (0.156–6.94 μg/ml of oil) except Syrian olive oil (310.67 μg/ml of oil). More remarkably, no C13:0, C14:0 and C15:0 FAs were detected in any oil samples (Table 4). The separation of eight FAs was achieved in less than 8 min, and the consumption of mobile phase for each sample was about 12 mL. To validate the results, the determination was repeated three times and the standard deviation was found in the range of 0.4–3.6 (Table 4).

4. Conclusions

A fast, simple, and sensitive SIR acquisition based UPHLC–MS method has been established for the simultaneous quantification of eight fatty acids in olive oils of various brands. The established approach predicts the composition of olive oils including saturation and unsaturation with good precision and give quantitative values for the acids content. Total eight olive oils were analyzed and the total fatty acids contents in each brand oils were found in the range of 472.63 (UK olive oil) to 7751.20 μg/ml (Syrian olive oil). Among the analyzed fatty acids, oleic acid was found to be the main fatty acid in all oils and the maximum contents of oleic acid (3785.94 μg/ml) was found in Syrian olive oil, while the smallest amount of oleic acid (229.30 μg/ml) was obtained in UK olive oil. In addition, the obtained quality parameters confirm that the current UPLC–MS method is sensitive, reproducible and simple and hence the approach allow to establish a quick screening method for the effective estimation of fatty acids in various oils and other matrices and it could have potential application for the determination of quality and authenticity of oils. Moreover, in the proposed method there is no need to prior derivatization of the FAs and the oil sample can be injected directly after dilution and filtration. The method is helpful to save a lot of time and cost compared to the other chemical methods as most of the reported method needs derivatization of the fatty acids before analysis.

Conflict of interest

The authors have declared no conflict of interest.

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