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Determination of alcohols in essential oils by liquid chromatography with ultraviolet detection after chromogenic derivatization

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ABSTRACT

An HPLC-UV method to determine compounds having a hydroxyl functional group in plant essential oils is developed. The sample is diluted with 1,4-dioxane and the analytes are derivatized with phthalic anhydride. The derivatives (phthalates hemiesters) are separated on a C8 column using an acetonitrile (ACN)/water gradient. Separation conditions were optimized using the DryLab® method development software. For the alcohols and phenols present in mint and rose essential oils, optimization led to a *ca.* 40 min gradient time and a column temperature of 8 °C. The alcohol and its derivatives were identified using HPLC with mass spectrometry (MS) detection. A large sensitivity enhancement was obtained by derivatization protocol. The HPLC-UV method was compared to GC with flame ionization detector (FID) and GC–MS. The limits of detection (LODs) obtained by the proposed method were better than those obtained by GC-FID and of the same order as those achieved by GC–MS. The three methods were satisfactorily applied to the determination of alcohols in essential oils. Therefore, the recommended method is of interest as an alternative to GC methods, to investigate the presence of compounds having an alcohol group at low concentrations in essential oils.

1. Introduction

Essential oils, which are obtained from plant parts by steam distillation, are used in a variety of applications, mainly in cosmetics, personal care products and in natural medicine [1]. Biosynthesized by a considerable number of aromatic plants, these oils are constituted by complex mixtures of a large variety of volatile substances, most of them showing a strong sensorial impact. These oils are predominantly constituted by monoterpene and sesquiterpene hydrocarbons, their oxygenated derivatives, a variety of aliphatic oxygenated compounds, and a few aromatic compounds [2]. Apart from its wide use in the flavor and cosmetic industries, several pharmacological (antimicrobial, antifungal, insecticidal, anthelmintic, antioxidant) properties have been reported [3–5]. Owing to the widespread industrial applications, its influence on human health and the defense of the consumer rights, methods for the quick and reliable characterization of essential oils are required.

Essential oils are usually analyzed by GC with flame ionization detector (FID), MS detection [6,7] and GC-olfactometry [8]. However, difficulties in the GC–MS peak identification of these complex samples due to the fact that many terpenes have identical mass spectra occur with some frequency [9]. This is a consequence of the close similarities, both in the initial molecule or in the fragmentation patterns and rearrangements after ionization. Therefore, GC–MS identification of these compounds is frequently confirmed by using the retention indexes [10]. In addition, structural alterations of thermally labile compounds may occur during GC due to the high injector temperatures or contact with the catalytically active surfaces of columns or liners [11]. For these reasons, alternative methods to GC, which avoid the use of high temperatures, and which could be used for reference, are of interest.

Owing to the volatility and low UV absorptivity of most of their components, essential oils have been scarcely analyzed by HPLC [6,12–20]. In fact, most HPLC reports dealing with essential oils have focused on the detection of the non-volatile fraction of citrus oils [6,14–16], sample cleaning or fractionation previous to GC [17]. However, HPLC has been used to detect specific terpenoids [18,19]. Recently, Turek and Stintzing [20] have reported an HPLC-UV-MS^2 method to characterize fingerprint of essential oils. The essential oils were characterized by their respective UV peak patterns, which were mainly due to the absorbent components.

Alcohols and a variety of multifunctional compounds also containing hydroxyl functional groups constitute an important part of essential oils [2]. Thus, among the 100 compounds most frequently reported in essential oils, 25 of them are aliphatic alcohols and 4 of them are esters of aliphatic alcohol residues with more than 4 carbon atoms [21]. Among the 49 components studied by Turek

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and Stintzing [20] in seven essential oils, 13 were aliphatic alcohols (a *ca.* 27%), any of them showing an absorbance maximum over 200 nm, and 3 of them were phenols.

To enable or to enhance the absorptiometry, fluorimetric or mass spectrometric response of aliphatic alcohols in the UV, pre-column derivatization with either a symmetric cyclic anhydride [22–25], an acyl chloride [26], an isocyanate [27] or another reagent [28,29], is frequently used. With pre-column derivatization, the determination of non-absorbing alcohols with low LODs in complex matrices is possible.

As far as we know, methods for essential oils based on the derivatization of alcohols and other active hydroxyl functional groups as phenols, followed by HPLC with UV or fluorometric detection of the derivatives have not been yet described. Nevertheless, this approach should provide sensitive methods, useful to characterize the alcoholic fraction of the essential oils, and an alternative to the predominant use of GC–MS methods. According to Babushok et al. [21], a *ca.* 25% of the most frequent components reported in essential oils are aliphatic alcohols. These compounds should give and enhanced response after derivatization of the alcohol group. In addition, phenols such as thymol, carvacrol or eugenol are also derivatized. These compounds showed a remarkable molar absorptivity in the UV; however, their LODs could be also improved after derivation. In this work, the aliphatic alcohols present in mint and rose essential oils were determined by HPLC-UV after esterification with phthalic anhydride. Samples of *Mentha Arvensis* and *M. Piperita*, and two commercial samples of *Mentha* and *Rose* essential oils, were studied. The advantages and limitations of the proposed methods are discussed. Figures of merit, including limits of detections, are evaluated and compared to those obtained by GC-FID and GC–MS.

On the other hand, traditional strategies for the development of robust HPLC methods to separate the components of complex samples require considerable skill and patience of the chromatographer. This labor intensive and time-consuming process is shortened and greatly facilitated by using a computer-assisted HPLC method development software [30]. Further, the chances of finding a true absolute optimum, rather than a secondary relative optimum, largely increase. In this work, DryLab® was used to optimize the HPLC separation conditions for the alcohol and phenol derivatives found in mint and rose essential oils. For this purpose, a small well-defined number of experiments were performed to predict the effect of variations of the mobile phase composition, gradient time and column temperature on the peak position and shape [31–33].

2. Experimental

2.1. Reagents and samples

Urea, phthalic anhydride and menthol from Fluka (Buchs, Switzerland), ammonium hydroxide from Panreac (Barcelona, Spain), 1,4-dioxane, HPLC grade methanol and acetonitrile (ACN) from Scharlab (Barcelona), and camphor, geraniol, β-citronellol, terpinene-4-ol, thymol, isopulegol, carveol, linalool and phenyl ethyl alcohol from Sigma–Aldrich (St. Louis, MO, USA) were used. Deionized water (Barnstead deionizer; Sybron, Boston, MA) was also used. Commercial essential oils of mint and rose from Al Fayed (Aswan, Egypt), and *M. arvensis* and *M. piperita* oils supplied by Guinama (Valencia, Spain) were also used.

2.2. GC procedures

The GC-FID analysis was performed with a Focus system equipped with an AI 3000 autosampler from Thermo Fisher Scientific (Austin, TX, USA). A TRB-5 capillary column (30 m, 0.32 mm i.d., 0.50 μm film thickness) from Teknokroma (Barcelona) was used. The GC oven temperature program was operated as follows: 50 °C (2 min), followed by rising to 250 °C at 20 °C min^{-1} with a final 250 °C plateau (5 min). The FID was set at 280 °C. The GC injection port was set at 250 °C; an injection volume of 10 μL was used under split mode (5:1). Nitrogen was used as a carrier gas at a constant flow rate of 1 mL min^{-1}.

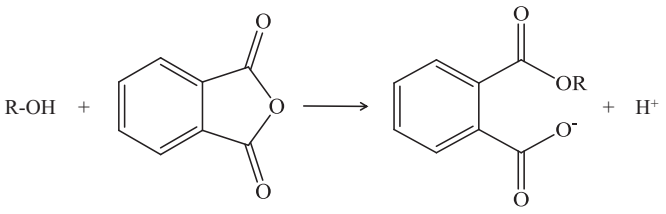

Fig. 1. Esterification of alcohols with phthalic anhydride.

The GC–MS analysis was performed on a Focus DSQ II gas chromatograph provided with an AI 3000 autosampler and single quadrupole MS detector from Thermo Fisher Scientific. The analytical fused-capillary column was a TR-5MS (30 m, 0.25 mm i.d., 0.25 μm film thickness) from Thermo Fisher Scientific. The GC oven temperature program was as indicated above. The injector, transfer line and ion source temperatures were set at 280 °C. The injection volume was 4 μL under splitless mode. Ionization was done by electron impact at 70 eV. Ions within the *m/z* 50–400 range were monitored. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. For GC-FID and GC–MS analysis, both standards and samples were diluted in hexane and injected. For quantitation studies, camphor was used as internal standard. Calibration curves of several alcohols up to 200 μg mL^{-1} were constructed.

2.3. Derivatization procedure

The derivatization procedure was adapted from literature [23]. The esterification reaction is depicted in Fig. 1. The derivatized alcohol compounds showed absorption maxima at 230 nm, giving high molar absorptivities comprised in the range 8770–11720 L mol^{-1} cm^{-1}. These values were consistent with those reported in the literature [34]. The esterification protocol was carried out as follows. Briefly, 0.2 g of essential oil or an aliquot of a solution of 5000 μg mL^{-1} of an alcohol standard was weighed. Then, 0.75 g of phthalic anhydride, 0.25 g of finely ground urea and 2 mL of 1,4-dioxane were added. The mixture was shaken in vortex and heated at 105 °C in a silicone oil thermostatic bath for 90 min. After cooling, the final volume was completed to 12 mL with a 2:1 MeOH/water containing 0.1 M NH_3. The solutions were properly diluted in ACN/water and injected immediately into the HPLC or kept at −20 °C until use. In all cases, satisfactory reaction yields (*ca.* 97–99%) were achieved.

2.4. HPLC separation of the alcohol derivatives

A 1100 series HPLC system equipped with a binary pump, a thermostatic column compartment, and a UV–Vis variable wavelength detector (Agilent Technologies, Waldbronn, Germany), was used. The separation was carried out with an Ascentis-Express C8 fused-core column (15 cm × 4.6 mm ID, 2.7 μm particle size) from Supelco (Bellefonte, PA, USA). ACN/water mobile phases containing 0.1% acetic acid were used. Gradient elution was carried out from 30 to 80% ACN. In all cases, the flow rate was 1 mL min^{-1}, 20 μL was injected, and all injections were performed by triplicate. The detection wavelength was 230 nm. In order to optimize the separation conditions using DryLab® (Molnár Institute, Berlin, Germany),

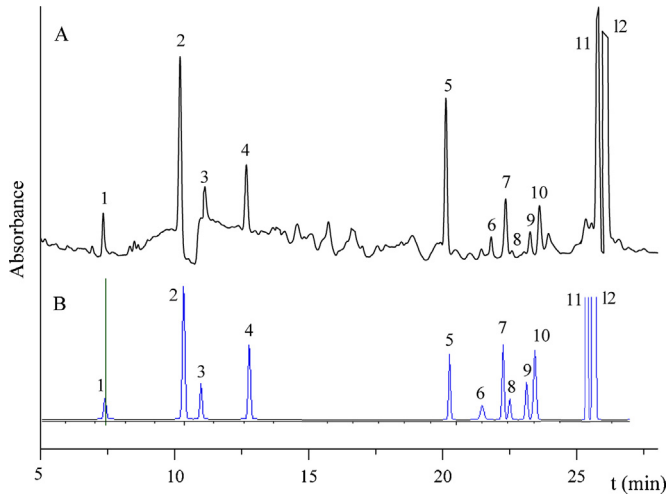

Fig. 2. Experimental (A) and predicted (B) chromatograms of the derivatized commercial (Al Fayed) mint essential oil obtained in the optimized separation conditions (from 30% to 80% ACN in 39 min at 8 °C). The 12 peaks marked with numbers were those included in the optimization of the separation by DryLab®.

the following initial set of four chromatograms were obtained. For each sample, two linear gradients, with gradient times of 20 and 40 min, were used, and for each gradient time, two chromatograms at two column temperatures, 10 and 25 °C, were obtained. The chromatograms were imported with the PeakMatch® software in AIA format (*.cdf) for "peak tracking", which refers to the matching of peaks of the same compound between runs where conditions have changed. After exporting, peaks areas were compared. Very small peak areas were not included in the studies that follow. The resulting retention times and peak areas of individual peaks were used as input data for method development with DryLab®.

For HPLC-MS experiments, the liquid chromatograph was coupled (in series with the UV–Vis detector) to the ESI source of a high speed Triple TOF™ 5600 mass spectrometer (AB SCIEX, Concord, Canada). The Triple TOF working conditions were: ion spray voltage floating, 5500 and −4500 V for positive and negative-ion modes, respectively; curtain gas, 25 psi, interface heater temperature, 450 °C; ion source gas 1 and 2, 50 psi. All data were acquired using the information-dependent acquisition (IDA) mode with Analyst TF 1.5 software (AB SCIEX). For IDA parameters, 0.25 s MS survey scan in the mass range of *m/z* 50–650 were followed by 30 MS^2 scans of 50 ms in the mass range of 50–650 (total cycle time, 1.75 s).

3. Results and discussion

3.1. Optimization of the HPLC separation of derivatized alcohols in mint and rose essential oils

A chromatogram of a derivatized sample of commercial (Al Fayed) essential mint oil is shown in Fig. 2A. This chromatogram, showing a complex peak pattern, was obtained after optimization of the separation conditions using DryLab®. Only the peaks with a significant signal-to-noise ratio were included in the optimization of the separation conditions. The chromatograms obtained before optimization (not given) showed a few well-resolved peaks and some groups of partially resolved peaks. Care was taken of recognizing all the peaks of these groups to be individually included in DryLab® for separation optimization. Additionally, the peaks of the reagent blank, which were located at the head of the chromatogram, were excluded. The selection process resulted in a set of 12 peaks. Then, DryLab® was used to optimize both the gradient time and the column temperature. To design the initial set of experiments, the following was taken into account: (a) the initial and final ACN concentrations, 30% and 80%, respectively, were selected according to the literature related to the HPLC separation of phthalates of alcohols with $8 < n < 18$ carbon atoms [23,25]; (b) for convenience, the minimal and maximal gradient time was set at 20 and 40 min, respectively; c) the column temperature was allowed to vary from 5 to 25 °C. From the initial set of four chromatograms, DryLab® predicted the peak locations in all intermediate working conditions, also calculating the resolution between the successive peak pairs. From these, a resolution map with red areas corresponding to the highest resolution of the most critical peak pair (R_C), was provided. The resolution map for the derivatized sample of Al Fayed mint essential oil is shown in Fig. 3. The best resolution was predicted for a 39 min gradient time at a column temperature of 8 °C. As shown in Fig. 2, the chromatogram obtained in these conditions (Fig. 2A) satisfactorily matched with the predicted chromatogram (Fig. 2B). The experiments, which follow were performed in these conditions.

The peak retention times and resolutions for the predicted and experimental chromatograms, obtained in the optimized conditions, are given and compared in Table 1. The average relative errors in the retention times and resolutions were 1.2% and 19.1%, respectively. Similar levels of accuracy for DryLab®, in terms of the predicted retention times, have been recently reported [35,36]. The prediction errors were much larger for resolutions than for retention times, but this agrees with other reported values [36]. It should be noted that the errors of predicted resolutions depend on both the retention time errors and the uncertainties of peak width and symmetry [36].

Samples of *M. Arvensis* and *M. Piperita*, and the Al Fayed sample of rose essential oil, were also derivatized as indicated, and aliquots were injected. For each essential oil, the initial set of four chromatograms was obtained, and optimization of the separation conditions with Drylab® was performed. For a given set of HPLC conditions, the chromatograms of the derivatized *M. Arvensis* and *M. Piperita* oils were closely similar to those obtained with the Al Fayed mint essential oil. Therefore, the resolution maps for the three mint oils predicted the same set of optimal conditions, *i.e.* a 39 min gradient time and 8 °C. Concerning to the rose essential oil; a total of eight peaks were selected for optimization. The best resolution of the most critical peak pair was predicted for a 37 min gradient time at 6 °C (resolution map not shown); however, for simplicity, we decided to use the optimal conditions obtained for the mint essential oils (39 min gradient time and 8 °C) also for the rose essential oil, since the resolution of the most critical peak pair ($R_C = 1.34$) was only slightly lower than that obtained in the optimal conditions ($R_C = 1.51$). Predicted and experimental chromatograms of a sample of derivatized rose essential oil, obtained in these conditions, are given in Fig. 4A and B, respectively. For the rose essential oil, in these conditions, retention times and resolutions were predicted with average errors of 1.3% and 29.1%, respectively.

3.2. Identification of underivatized and derivatized alcohols in essential oils using HPLC-MS

In order to identify the chromatographic peaks (Figs. 2 and 4), MS detection was also coupled in series with the UV–Vis detection. Chromatograms of both alcohol standards and the Al Fayed mint and rose essential oils, without and with previous derivatization with phthalic anhydride, were obtained. All chromatograms were obtained in the optimal conditions for the derivatized mint essential oil. The total ion chromatograms (TICs) and several extracted ion chromatograms (EICs) of the same mint essential oil, without and with previous derivatization with phthalic anhydride, are shown in Fig. 5A and B, respectively. A reagent blank, prepared by using the derivatization procedure in the absence of a sample,

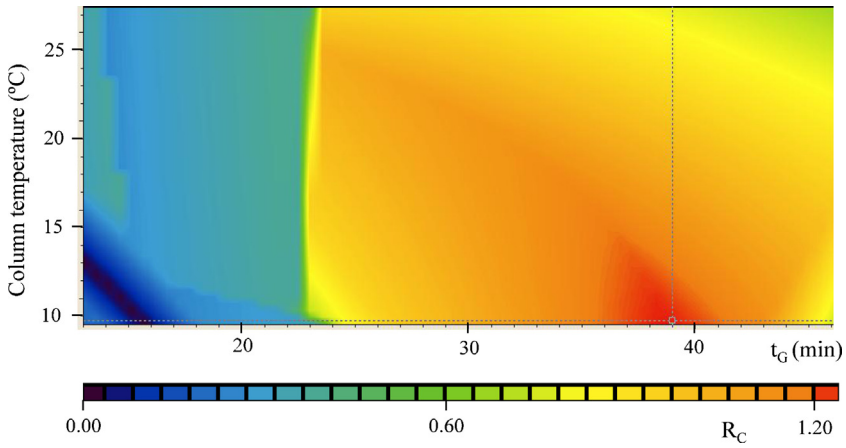

Fig. 3. Two-dimensional resolution map of gradient time *versus* column temperature for the derivatized Al Fayed mint essential oil. Blue and red areas correspond to low and high resolutions of the most critical peak pair, respectively. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

Table 1
Predicted and experimental retention times and resolution data for the optimized separation of derivatized compounds present in a commercial mint essential oil.

Peak	Retention time				Resolution			
	Experimental	Predicted	Diff.[a]	% Error[b]	Experimental	Predicted	Diff.[a]	% Error[b]
1	7.26	7.38	−0.12	1.6	8.76	10.90	−2.13	19.5
2	10.13	10.30	−0.17	1.6	3.51	2.75	−0.76	27.7
3	11.07	10.95	0.12	1.1	5.21	8.57	−3.36	39.2
4	12.56	12.74	−0.18	1.4	26.04	28.50	−2.46	8.6
5	20.00	19.52	0.48	2.4	7.00	6.00	1.00	16.7
6	21.67	21.31	0.36	1.7	1.80	2.73	−0.93	34.0
7	22.20	22.20	0.00	0.0	0.90	0.75	0.14	18.5
8	22.44	22.38	0.06	0.3	3.14	2.86	0.29	10.0
9	23.10	22.98	0.12	0.5	1.33	1.00	0.33	33.3
10	23.45	23.27	0.18	0.8	6.55	6.40	0.15	2.3
11	25.60	25.18	0.42	1.7	1.20	1.20	0.00	0.0
12	25.95	26.54	0.42	1.6				
Average standard error		1.2				19.1		

[a] Difference = Experimental − Predicted.
[b] % Error: [(Experimental − Predicted)/Predicted] × 100.

was also chromatographed. This chromatogram showed that the reagent peaks were eluted at the head of chromatogram, thus causing no interference (not shown). For the underivatized essential oils, satisfactory signals from positive quasimolecular ions $[M+H]^+$ [20,37,38] were obtained at the retention times of several sample components. As shown in Table 2, the *m/z* values agreed with the expected values for several compounds, which are common components of the mint and rose essential oils, and which also have an alcohol functional group. MS^2 data was also used to confirm peak identifications. In particular, the *m/z* differences between several MS^2 intense peaks corresponding to loss of water, and several easily recognizable fragments, were useful [37,38]. For the mint essential oils, five from twelve peaks; which were selected for HPLC optimization (see Fig. 3), were identified. The identification was confirmed by injecting the alcohol standards. These gave the same MS and MS^2 spectra at the same retention times as the target

Table 2
Chemical formula, parent ion and fragments of underivatized and derivatized alcohols found in the mint and rose essential oils.

Peak label	Compound	Formula	ESI (+)-MS (*m/z*)[a]	MS^2 (+) (*m/z*)	Derivative formula	ESI (−)-MS (*m/z*)[b]	MS^2 (−) (*m/z*)
Mint oils							
2	Terpinene-4-ol	$C_{10}H_{18}O$	155	137, 107	$C_{10}H_{18}O$—$C_8H_4O_3$	301	257, 165, 153, 135
4	Thymol	$C_{10}H_{14}O$	151	135, 121	$C_{10}H_{14}O$—$C_8H_4O_3$	297	253, 165, 149, 133
5	Isopulegol	$C_{10}H_{18}O$	155	137, 83	$C_{10}H_{18}O$—$C_8H_4O_3$	301	257, 165, 154, 135
11	β-Citronellol	$C_{10}H_{20}O$	157	137, 83	$C_{10}H_{20}O$—$C_8H_4O_3$	303	259, 165, 155, 135
12	Menthol	$C_{10}H_{20}O$	157	137, 83	$C_{10}H_{20}O$—$C_8H_4O_3$	303	259, 165, 155, 135
Rose oil							
13	Terpinene-4-ol	$C_{10}H_{18}O$	155	137, 107	$C_{10}H_{18}O$—$C_8H_4O_3$	301	257, 165, 153, 135
14	Carveol	$C_{10}H_{16}O$	153	135, 107	$C_{10}H_{16}O$—$C_8H_4O_3$	299	255, 165, 151, 133
15	Phenylethyl alcohol	$C_8H_{10}O$	123	105, 95	$C_8H_{10}O$—$C_8H_4O_3$	269	225, 165, 121, 93
17	Linalool	$C_{10}H_{18}O$	155	137, 95	$C_{10}H_{18}O$—$C_8H_4O_3$	301	257, 165, 153, 135
18	Geraniol	$C_{10}H_{18}O$	155	137, 95	$C_{10}H_{18}O$—$C_8H_4O_3$	301	257, 165, 153, 135
20	β-Citronellol	$C_{10}H_{20}O$	157	137, 83	$C_{10}H_{20}O$—$C_8H_4O_3$	303	259, 165, 155, 135

[a] Underivatized essential oils, *m/z* values for $[M+H]^+$.
[b] Derivatized (phthalates) essential oils, *m/z* values for $[M-H]^-$ (ionized hemiesters).

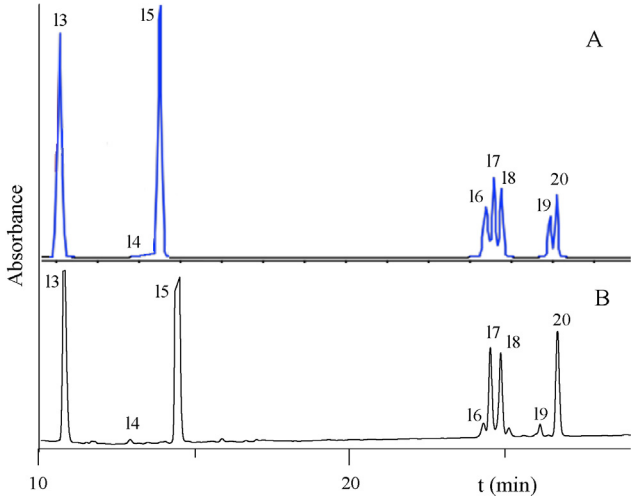

Fig. 4. Experimental (A) and predicted (B) chromatograms of the derivatized Al Fayed rose essential oil obtained in the optimized conditions of Fig. 2. The 8 peaks marked with numbers were those included in the optimization of the separation by DryLab®.

compounds. Evidence of the presence of these alcohols in the mint essential oils was subsequently confirmed by GC–MS (Section 3.3).

For the derivatized essential oils, the ESI was operated in the ion-negative mode. As also shown in Table 2, the intense signals of the $[M-H]^-$ ions of the phthalate esters of the same alcohols observed

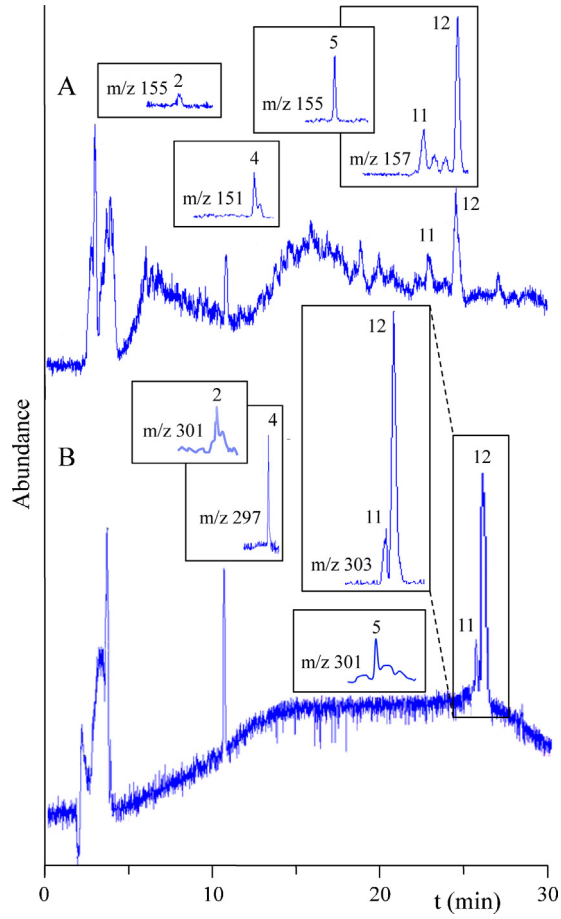

Fig. 5. TIC and EICs of a sample of underivatized (A) and derivatized (B) Al Fayed mint essential oil. Peak labeling according to Table 2. The EICs obtained at some of the *m/z* values indicated in Table 2 are shown.

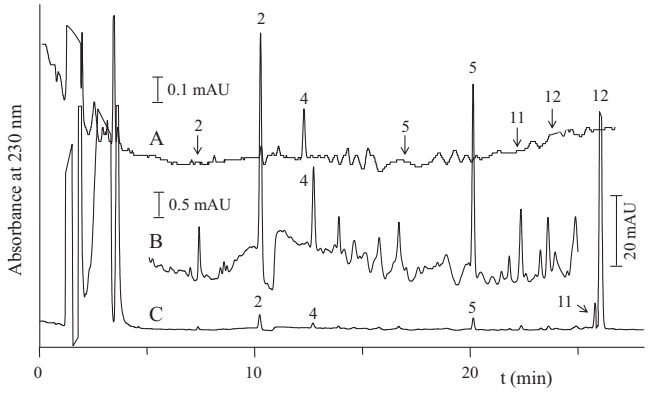

Fig. 6. HPLC-UV chromatograms of the underivatized (A) and derivatized (B and C) Al Fayed mint essential oil. The trace B is an expanded view of trace C. The arrows shown in trace A indicate the retention times of the underivatized alcohol components, as deduced from the TIC and EICs. Other details are indicated in Fig. 5.

with the underivatized oils were obtained. The MS^2 fragments of the hemiesters were also used to support peak identification [39,40]. The predominant fragment ions were deprotonated phthalic acid (*m/z* 165), and the alcohol residues (as $[M-H]^-$), obtained by cleavage of the ester bond, as well as fragments of the alcohol residue (Table 2) [39,40]. Using the information provided by MS detection, the peaks on the chromatograms obtained with UV detection were identified. In Fig. 6A, a UV chromatogram of an underivatized sample of mint essential oil is shown. The peak of the only component which absorbs at 230 nm, thymol, was observed on this chromatogram. The arrows on chromatogram indicate the retention times of the compounds of Table 2, which were identified by HPLC-MS/MS^2. In Fig. 6B, the UV chromatogram of the derivatized sample of the same mint essential oil is given. The predominant peak of menthol (peak no. 12), and the peaks of the other four compounds (given in Table 2) were identified. By comparing the chromatograms of Fig. 6A and B, it is deduced that the alcohol derivatives appeared in the same elution order than the corresponding underivatized alcohols, but with larger retention times, which could be explained by the presence of the phthalate moiety. The HPLC-UV-MS chromatograms of underivatized and derivatized rose essential oil were also obtained. Based on the MS data given in Table 2 (columns 4 and 7), up to six peaks corresponding to several alcohols (out of the eight peaks initially recognized for separation optimization) were identified.

3.3. Quantitation studies

The proposed HPLC-UV method was evaluated in terms of precision, linearity and sensitivity (limit of detection). Retention time and peak repeatabilities, and limits of detection (LODs) at a signal-to-noise ratio of S/N = 3 were obtained (Table 3). Intra- and inter-day repeatabilities were obtained by injecting a standard mixture containing 1 $\mu g\,mL^{-1}$ of each analyte three times per day over 3 days. External calibration curves were linear over the range 200 $\mu g\,mL^{-1}$, with correlation coefficients better than $r = 0.998$. Standard addition calibration curves were also obtained by adding to the essential oils three solutions with increasing alcohol concentrations up to 100 $\mu g\,mL^{-1}$. The curves were linear with $r > 0.999$, and in all cases the sensitivity was the same as with the external calibration method.

GC with FID and MS detection was also applied for qualitative and quantitative analyses of alcohols in the underivatized essential oils. The presence of alcohol peaks was confirmed by retention index and MS data, and by injecting alcohol standards.

Table 3
Significant analytical parameters of the proposed HPLC-UV method and a comparison with the LODs obtained by GC methods.

Analyte	t_R (%)[a]	Area (%)[a]	LOD HPLC-UV ($\mu g\,mL^{-1}$)	LOD GC-FID ($\mu g\,mL^{-1}$)	LOD GC–MS ($\mu g\,mL^{-1}$)
Terpinene-4-ol	0.11; 0.37	1.37; 2.91	0.08	1.1	0.07
Thymol	0.09; 0.33	2.12; 3.37	0.08	0.9	0.05
Isopulegol	0.05; 0.31	1.53; 3.14	0.021	1.1	0.06
β-Citronellol	0.03; 0.37	1.83; 2.92	0.08	1.2	0.06
Menthol	0.13; 0.34	1.50; 3.05	0.17	1.1	0.08
Carveol	0.17; 0.33	1.39; 3.22	0.19	1.0	0.10
Phenyl ethyl alcohol	0.08; 0.42	1.13; 2.97	0.20	1.1	0.09
Linalool	0.12; 0.31	1.46; 3.01	0.15	1.0	0.13
Geraniol	0.02; 0.34	1.87; 3.21	0.18	1.0	0.020

[a] As intra- and inter-day relative standard deviations.

Table 4
Alcohol concentrations (% w/w) found by HPLC-UV of the alcohol phthalates, GC-FID and GC–MS in the mint essential oils.

Analyte (%)	Al Fayed mint			*M. Arvensis*			*M. Piperita*		
	HPLC-UV	GC-FID	GC–MS	HPLC-UV	GC-FID	GC–MS	HPLC-UV	GC-FID	GC–MS
Terpinene-4-ol	0.20 ± 0.02	0.22 ± 0.01	0.26 ± 0.01	0.44 ± 0.07	0.47 ± 0.05	0.42 ± 0.03	0.21 ± 0.03	0.19 ± 0.02	0.22 ± 0.01
Thymol	0.08 ± 0.01	0.09 ± 0.02	0.11 ± 0.01	2.11 ± 0.31	2.07 ± 0.22	2.08 ± 0.17	0.17 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Isopulegol	0.06 ± 0.02	0.05 ± 0.01	0.07 ± 0.01	0.14 ± 0.06	0.13 ± 0.05	0.16 ± 0.02	–	–	–
β-Citronellol	0.33 ± 0.08	0.28 ± 0.05	0.29 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.31 ± 0.07	0.32 ± 0.04	0.29 ± 0.02
Menthol	17.81 ± 1.10	16.79 ± 0.87	17.55 ± 0.54	25.72 ± 0.85	24.97 ± 0.92	26.51 ± 0.73	15.87 ± 0.92	16.13 ± 0.67	15.72 ± 0.48

The calibration curves were linear over the range 1–200 $\mu g\,mL^{-1}$ for GC-FID and GC–MS, with correlation coefficients better than $r = 0.999$. The LODs (S/N = 3) are shown in the last two columns of Table 3.

Next, alcohol components of the mint and rose essential oils were quantified by using the proposed HPLC-UV method, and the results were compared with those obtained with GC-FID and GC–MS (Tables 4 and 5). The HPLC-UV and GC procedures yielded similar values for all the analytes, with no significant differences at the 95% confidence level. As shown in Table 4, menthol was the major constituent of all the mint essential oils, which was in agreement with literature [41], whereas phenyl ethyl alcohol (Table 5) was the main alcohol present in rose essential oil [42,43].

The HPLC-UV method was compared to the GC procedures in terms of chromatographic performance. Sample preparation for GC is straightforward, whereas derivatization is required for HPLC. There is also abundant literature describing the GC separation of essential oil components, whereas HPLC-UV literature related to the subject is scarce. Further, method development is more complex in HPLC than in GC; however, the use of modeling software like DryLab® reduced significantly the effort and time required for separation optimization. Regarding sensitivity, HPLC-UV of the alcohol phthalates has yielded LODs closely similar to those achieved by GC–MS, and better than those obtained by GC-FID (Table 3). In addition, alcohol derivatization followed by HPLC-UV is more selective than GC for the target analytes. Concerning analysis time, the drawback of derivatization is the time required to prepare the derivatives; however, in routine analysis much time can be saved by simultaneously derivatizing many samples. Also, methods for quick sample derivatization using off-line and on line [27,44] microwave irradiation, have been described. Concerning the separation time, HPLC and GC are similar. Finally, a drawback of GC is the need for volatility of the analytes, which limits the range of compounds that can be analyzed. In addition, structural alterations of thermally labile compounds may occur during GC analysis. The proposed HPLC-UV method constitutes an alternative, which can be useful to confirm or to extend GC studies. It is also complementary to the direct analysis of underivatized essential oils by HPLC-UV, since the alcohol components have low molar absorptivities [20]. Derivatization of alcohols is also useful in HPLC-ESI-MS, since aliphatic alcohols usually give low sensitivities [23]. Finally, phthalic anhydride has been used in this work, but substantial improvements of the LODs can be achieved using diphenic anhydride [23] or other reagents providing higher absorptivities or enabling fluorimetric detection.

Table 5
Alcohol concentrations (%, w/w) found by HPLC-UV of the alcohol phthalates, GC-FID and GC–MS in the Al Fayed rose essential oil.

Analyte (%)	HPLC-UV	GC-FID	GC–MS
Terpinene-4-ol	0.75 ± 0.11	0.77 ± 0.08	0.77 ± 0.04
β-Citronellol	1.07 ± 0.23	1.11 ± 0.12	1.04 ± 0.06
Carveol	0.03 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
Phenyl ethyl alcohol	4.53 ± 0.62	4.61 ± 0.54	4.57 ± 0.33
Linalool	0.59 ± 0.07	0.61 ± 0.10	0.61 ± 0.05
Geraniol	0.68 ± 0.17	0.65 ± 0.11	0.68 ± 0.05

4. Conclusions

A HPLC-UV method for the determination of alcoholic fraction of essential oils, based on their pre-column derivatization with an aromatic anhydride, has been developed. Quick and safe optimization of the chromatographic separation conditions for the alcohol fraction of mint and rose essential oils was achieved with the DryLab® method development software. The compounds found were identified using HPLC-MS of both derivatized and underivatized samples, and also by comparison to the GC-FID and GC–MS traces of the underivatized samples. The derivatized analytes provided a dramatic increase in UV absorption. Thus, the LODs obtained with the proposed method were similar to those obtained by GC–MS, and clearly better than those achieved by GC-FID. Then, the proposed method constitutes a good alternative to characterize and quantify components having an alcohol group in essential oils.

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