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Technical Report

Oxygen heterocyclic compounds profile evaluation in cold-pressed *Citrus* essential oils using supercritical fluid chromatography coupled to photodiode array detector

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Abstract:

A large number of analytical techniques to characterize the non-volatile composition of *Citrus* essential oils (EOs) were introduced in the literature. However, the reports of *Citrus* EOs analysis by supercritical fluid chromatography (SFC) with UV or mass spectrometry detectors for the analysis of non-volatile polar compounds are still few. The aim of the present scientific research is to describe a new analytical approach using SFC with a green mobile phase for the analysis of such compounds in cold-pressed *Citrus* EOs, to confirm the potential use of SFC-UV for oil classification in the context of quality control of raw materials in cosmetics. Gradient conditions are determined to achieve a satisfactory separation in 10 minutes.

Keywords: Oxygen heterocyclic compounds, *Citrus* essential oils, supercritical fluid chromatography, furocoumarins

1. Introduction

Citrus EOs are cold-extracted from the peel of *Citrus* fruits using different mechanical systems and are composed by a volatile fraction (85–99% of the *Citrus* oils) and a non-volatile fraction (1–15%). Coumarins, psoralens, and polymethoxyflavones are a class of oxygen heterocyclic compounds present in the non-volatile fraction of cold-pressed *Citrus* EOs. It is well known that psoralens exhibit strong photoactivity in combination with UVA radiation [1] and for this reason, there are opinions and regulations on the use of essential oils in cosmetic products [2-4]. The study of the non-volatile fraction of cold-pressed *Citrus* EOs is a valid tool to exploit their quality and authenticity, and it also represents an important means for product control in the cosmetic industry. RP-HPLC represent, from 2000 up to now, the most used technique for the analysis of oxygen heterocyclic compounds with optimal results in terms of accuracy, repeatability, limit of detection, and quantification [5]. In perspective of green chemistry, supercritical fluid chromatography (SFC) was not sufficiently exploited, more investigation using this analytical technique are needed.

The present research is focused on the development of a rapid analytical method with low environmental impact using SFC coupled to a photodiode detector (PDA), to analyze oxygen heterocyclic compounds in cold-pressed *Citrus* EOs. The separation of all compounds was achieved in less than 10 min, using a HILIC stationary phase and a mobile phase including CO_2 and ethanol with 0.6% of H_2O and 0.07% of trifluoroacetic acid (TFA).

Supercritical Fluid Chromatograph Nexera™UC

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2. Experimental

2-1. Samples, chemicals and materials

Seven samples of cold-pressed *Citrus* EOs (lime, lemon, bergamot, grapefruit, sweet and bitter oranges, and red mandarin) were analyzed by SFC-PDA method. All the solvents were provided by Merck KGaA, Darmstadt, Germany, except when otherwise specified. Water (LC-MS grade, purity ≥ 99.9%) LiChrosolv®, ethanol (EtOH, gradient grade for HPLC, purity ≥ 99.9%) LiChrosolv® and trifluoroacetic acid (eluent additive for LC–MS, purity ≥ 99.0%) were used for SFC-PDA analyses. Hexane (for LC, purity ≥ 98.0%) LiChrosolv® was used to prepare sample solutions. Cold-pressed *Citrus* EOs were analyzed without any pretreatment (each oil was diluted 1:1000 (v/v)).

2-2. SFC-PDA Shimadzu Nexera UC system

The separation and detection of the oxygen heterocyclic compounds was carried out by using a SFC-PDA on a Shimadzu Nexera UC system consisting of a CBM-20A communication bus module, two LC-20AD$_{XR}$ dual plunger parallel-flow pumps, an LC-30AD$_{SF}$ CO_2 pump, a SFC-30A backpressure regulator, a DGU-20A$_{5R}$ degasser, a CTO-20AC column oven, a SIL-30AC autosampler, and a SPD-M20A detector. The analytical conditions used are summarized in Table 1.

Table 1: Analytical conditions for the analysis of oxygen heterocyclic compounds in cold-pressed *Citrus* EOs.

Column	Fused Core HILIC Column (150 x 2.1 mm, 2.7 µm)
Mobile phase	CO_2 (solvent A) and ethanol with 0.6% of H_2O and 0.07% of TFA (solvent B)
Gradient	0 min, 0% B; 1 min, 3% B; 4 min, 3% B; 6 min, 10% B
Flow rate	1.0 mL·min^{-1}
Injection volume	1 µL
Column oven	60°C
UV detection wavelength	315 nm
Back pressure	120 bar

3. Results and discussions

A fast separation has been achieved in less than 10 min. The advantages related to the present method are multiple, going to the ecological point of view, which is the main characteristic of the SFC approach, to the sensitivity of the method. The SFC-PDA method is then clearly suitable to be applied for the quality control of oxygen heterocyclic compounds in *Citrus* EOs. In Figure 1 are reported the SFC chromatograms of lemon and lime cold-pressed essential oils analyzed.

The developed SFC-PDA method showed to be a valid and environmentally friendly analytical approach for the analysis of 37 selected oxygen heterocyclic compounds in cold-pressed *Citrus* EOs, as reported in Table 2.

In Figure 2 is reported the AGREE graphic [6] of the RP-HPLC-PDA [7] and SFC-PDA analytical methods adopted for analysis the oxygen heterocyclic compounds in cold-pressed *Citrus* EOs. As can be seen from the figure, the present approach is greener respect to the previous RP-HPLC-PDA validated from our research group. The advantages of the SFC-PDA methods regarded: the volume of analytical waste generated per analysis (20 mL vs 1 mL), the energy consumed, the use of toxic reagents (methanol and tetrahydrofuran vs ethanol), and the use of reagents obtained from renewable sources (ethanol).

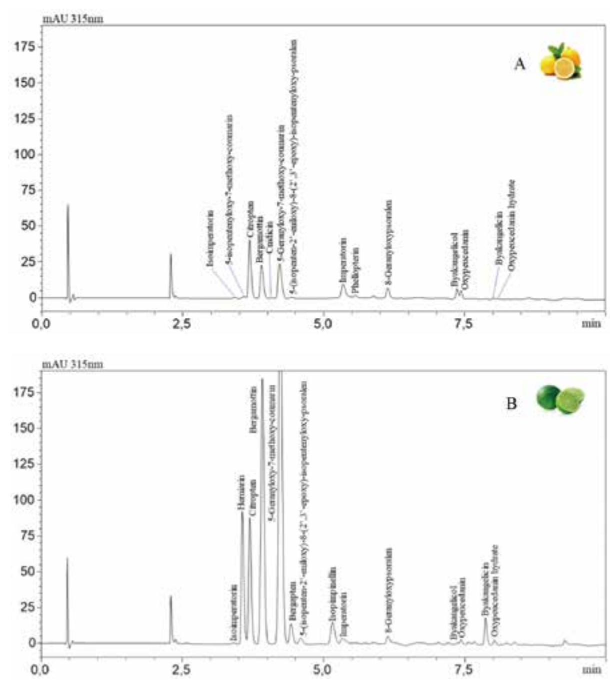

Figure 1: SFC-PDA chromatograms of lemon (A) and lime (B) cold-pressed essential oil.

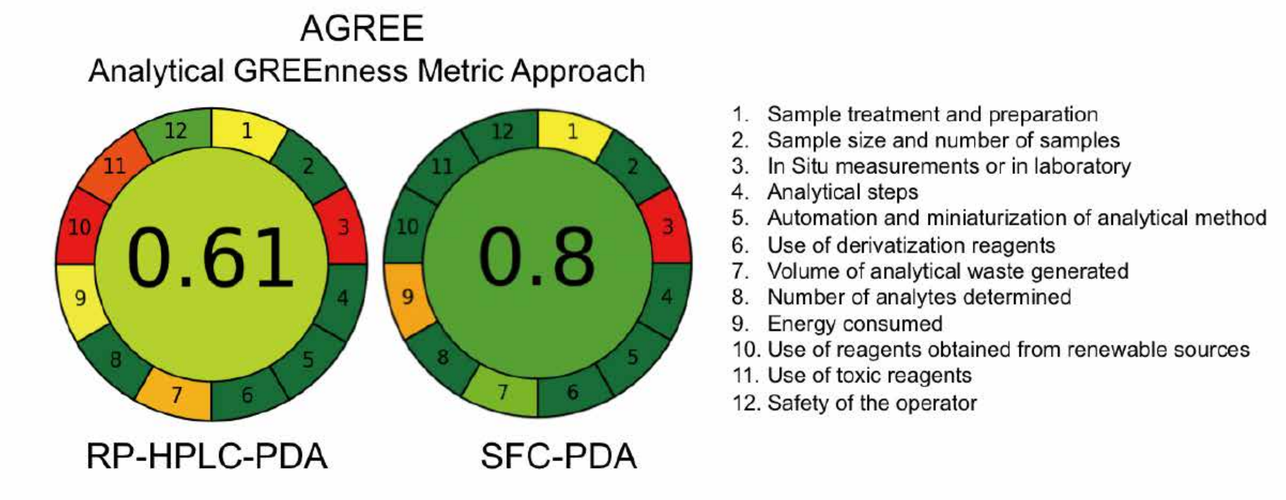

Figure 2: AGREE graphic of the RP-HPLC-PDA and SFC-PDA analytical methods adopted for the oxygen heterocyclic compounds analysis in cold-pressed *Citrus* EOs.

Table 2: Oxygen heterocyclic compounds identified in cold-pressed *Citrus* EOs investigated using SFC-PDA method.

#	**Compounds**	**Lime**	**Lemon**	**Bergamot**	**Grapefruit**	**Sweet orange**	**Bitter orange**	**Red mandarin**
1	Coumarin							
2	Isoimperatorin	x	x					
3	Herniarin	x		x				
4	5-Isopentenyloxy-7-methoxy-coumarin		x					
5	Citropten	x	x	x	x			
6	Bergamottin	x	x	x				
7	Cnidicin		x					
8	Cnidilin							
9	Psoralen							
10	5-Geranyloxy-7-methoxy-coumarin	x	x	x				
11	Bergapten	x		x	x		x	
12	Aurapten				x			
13	5-(isopenten-2′-eniloxy)-8-(2′,3′-epoxy)-isopentenyloxy-psoralen	x	x					
14	5-Geranyloxy-7-methoxy-psoralen							
15	Osthol				x			
16	8-Methoxypsoralen						x	
17	Isopimpinellin	x						
18	Heraclenin							
19	Imperatorin	x	x					
20	Phellopterin		x					
21	Epoxybergamottin				x			
22	Epoxyaurapten				x			
23	8-Geranyloxypsoralen	x	x					
24	Isomeranzin				x		x	
25	Meranzin				x		x	
26	Byakangelicol	x	x					
27	Oxypeucedanin	x	x					
28	Tangeretin				x	x	x	x
29	Byakangelicin	x	x					
30	Epoxybergamottin hydrate			x			x	
31	Oxypeucedanin hydrate	x	x					
32	Heptamethoxyflavone			x		x	x	x
33	Tetra-O-methyl-scutellarein			x		x		x
34	Nobiletin			x		x		x
35	Hexamethoxyflavone						x	
36	Meranzin hydrate			x			x	
37	Sinensetin			x		x		x

4. Conclusion

The separation of oxygen heterocyclic compounds in cold-pressed *Citrus* EOs is a challenge due to the wide variety of compounds and due to small structural differences between the compounds. The results obtained show that SFC-UV is a perfectly suited method to investigate the essential oil composition, because of the great number of compounds separated in a reduced analysis time (around 10 min), and with a very short time for re-equilibration of the system at the end of the gradient analysis.

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