

# STEROLS ANALYSIS IN OLIVE OIL BY MICROWAVE-ASSISTED SAPONIFICATION AND EXTRACTION FOLLOWED BY FLOW MODULATION COMPREHENSIVE TWO DIMENSIONAL CHROMATOGRAPHY

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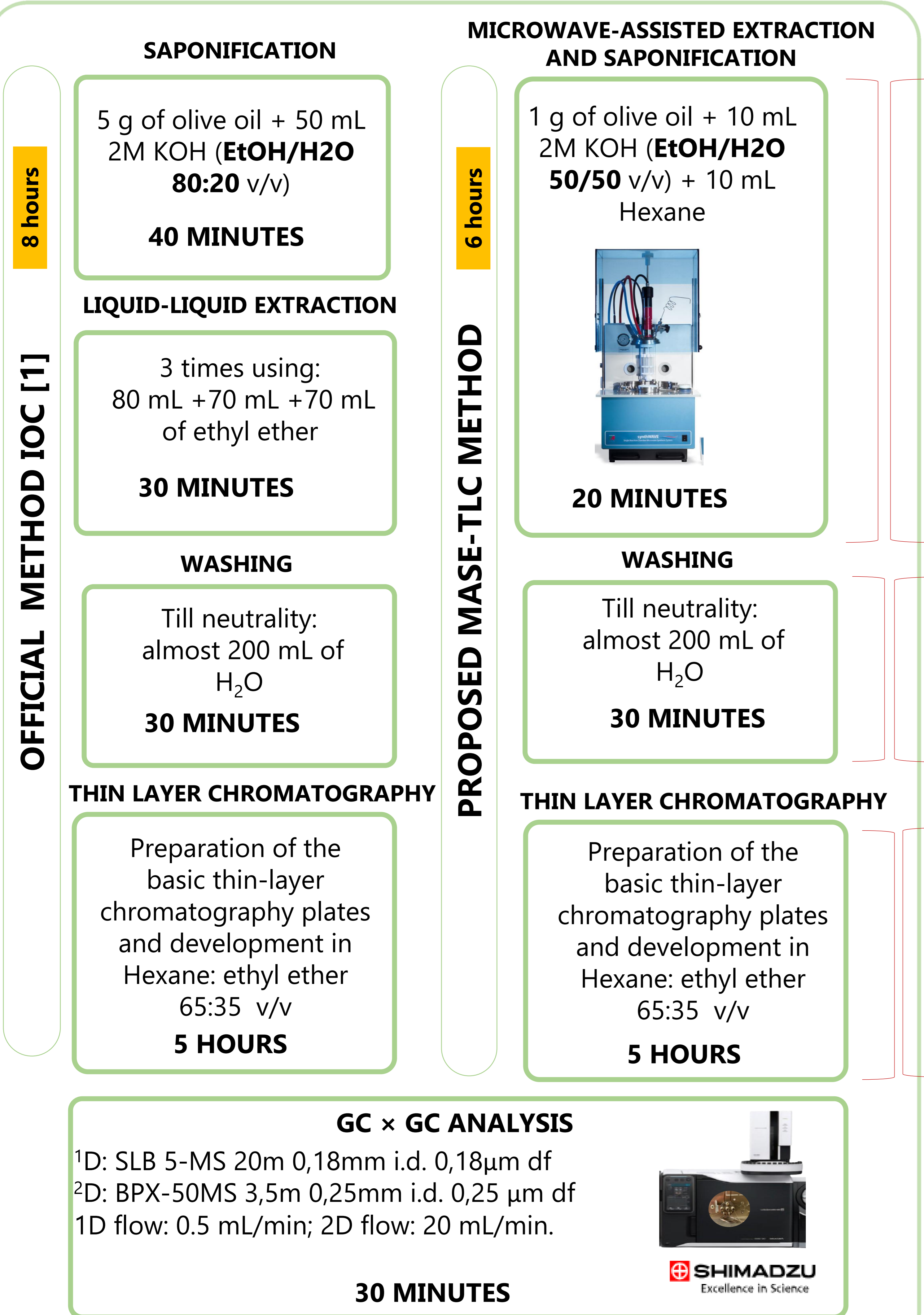
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## INTRODUCTION

The official method proposed by the International Olive Council [1] analyzes the sterol fraction through a multi-step process: initial saponification followed by liquid-liquid extraction and isolation of the fraction using thin-layer chromatography (TLC). The final analysis is carried out with gas chromatography coupled with a flame ionization detector (GC-FID). To streamline and accelerate this process, the sample is processed using a Microwave-Assisted Saponification and Extraction (MASE) method, with purification achieved through solid-phase extraction (SPE) prior to analysis in one-dimensional chromatography (1D GC). Despite these improvements, this study focuses on replacing the 1D GC method with comprehensive two-dimensional gas chromatography (GC×GC) to enhance the separation of sterol components.

## METHOD & MATERIAL



## PERSPECTIVE

Planned  
Replace extraction solvent: from hexane to Cyclohexane.  
Also changing the proportion EtOH/H<sub>2</sub>O from 50:50 to 20/80

Reducing washing time and amount of water

Replace Thin layer chromatography with Solid phase extraction (SPE) to reduce time and amount of solvents.

Some progress are presented, but in general is a



## RESULTS & DISCUSSION PART 1

Figure 1. Shows the GC×GC chromatogram of sterols in Extra Virgin Olive Oil (EVO). All the compounds are well separated, that consents a reliable quantification of sterols.

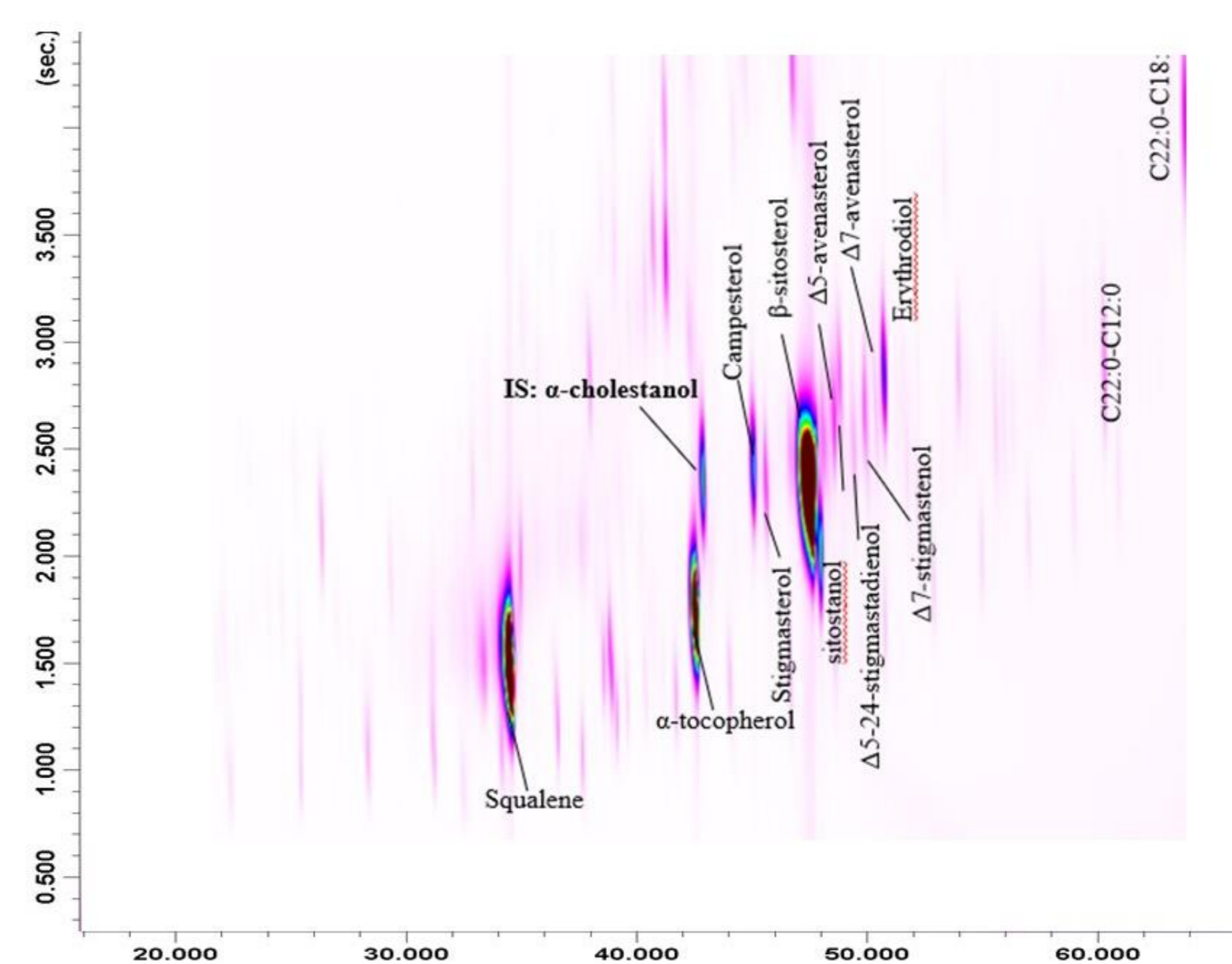


FIGURE 1.

The technique also allow the "diagnosis" of an adulterated olive oil. Figure 2. shows the comparison of sterols in EVO (a)

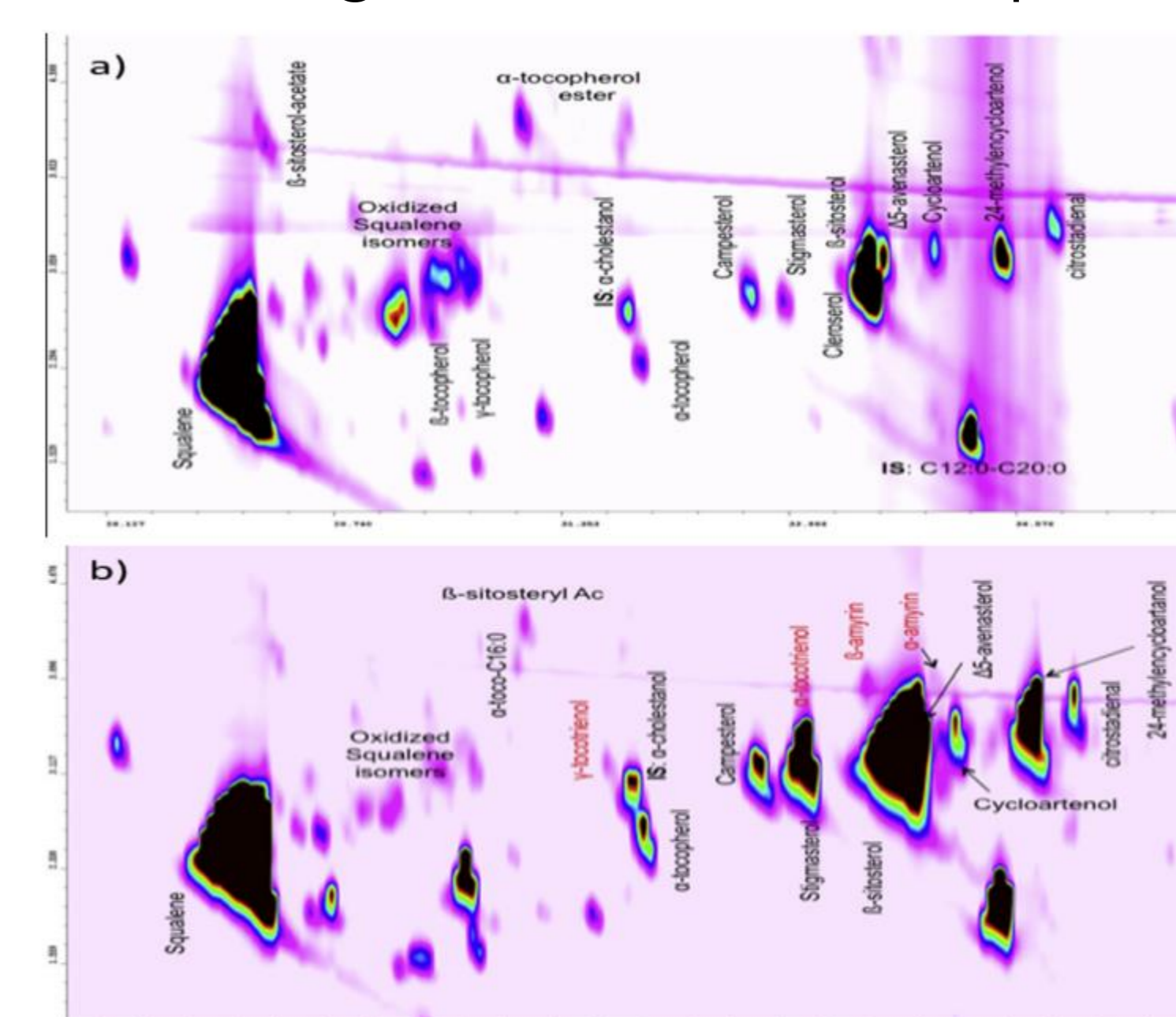


FIGURE 2.

And a mix of 90% EVO + 5% of High oleic sunflower oil (HOSO)+5% palm oil (PO).

In Figure 2b key diagnostic sterol compounds (absent in the EVO sample but abundant in PO and HOSO) were detected in the mixture.

Thanks to TLC separation, sterols are well separated from compounds that could interfere with their identification and quantification. However, this technique is highly time-consuming, and to enhance the outcome of this analysis it is necessary to improve this step. HOW to do it is discussed in Results and Discussion Part 2.

## CONCLUSION & FUTURE PERSPECTIVE

The optimized MASE method proposed a faster solution for the sample preparation in the analysis of sterols. The overall procedure is under further optimization. In particular, the step of SPE to further reduce volumes and minimize the co-elution of sterols and desmethyl sterols will be optimized. Keeping the big advantages of GC × GC for the chromatographic analysis.

## REFERENCES

[1] International Olive Council (IOC). International Olive Council: Madrid, Spain, 2017. [2] Mascrez et al Foods 2021 Feb 18;10(2):445

## ACKNOWLEDGEMENT

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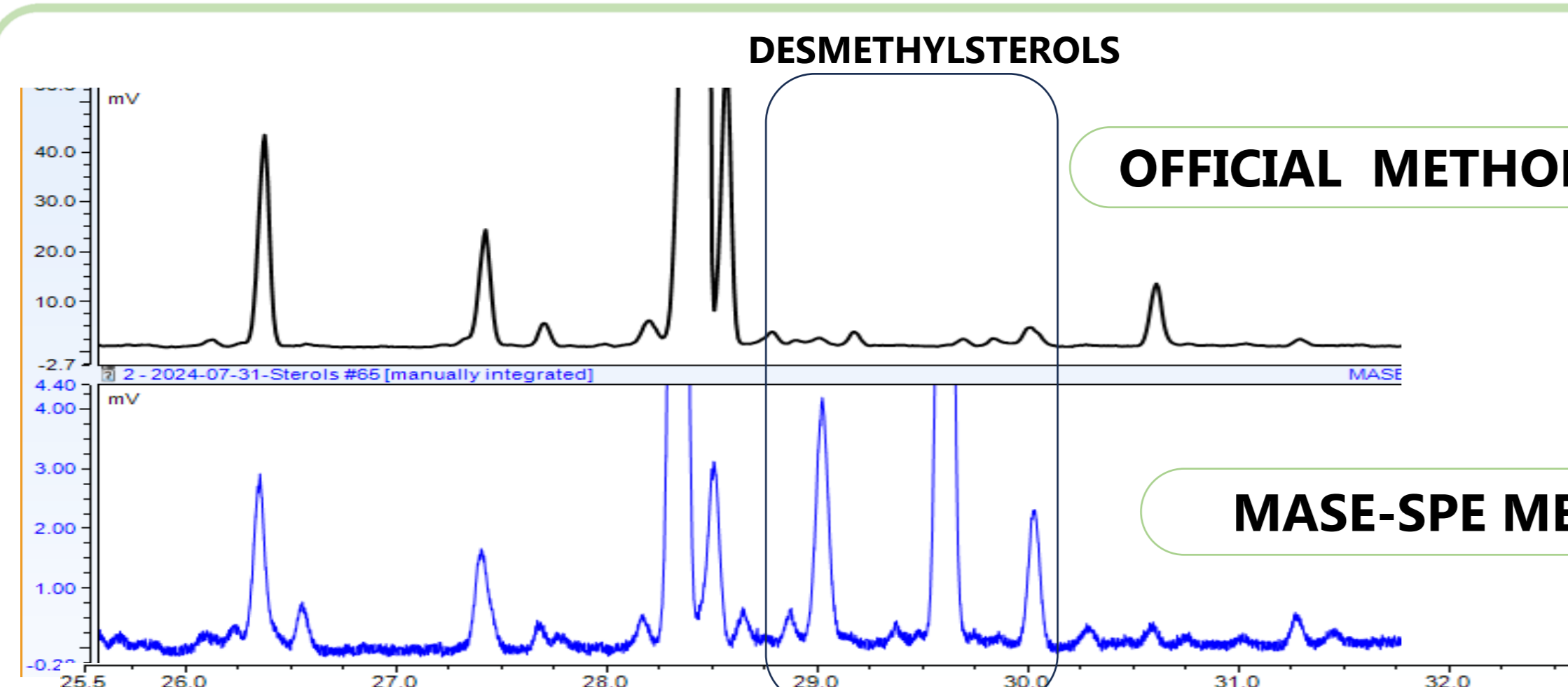
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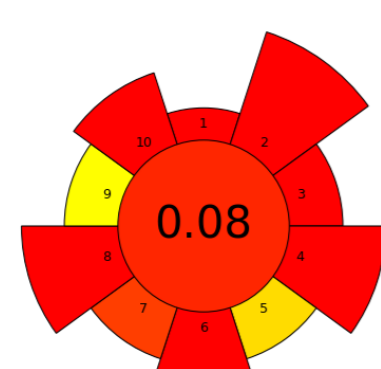
## RESULTS & DISCUSSION PART 2



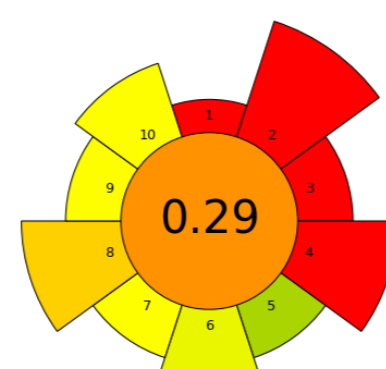
To replace TLC, we aim to use SPE. A study by Mascrez et al [2]. proposes an SPE method involving the purification of the unsaponifiable fraction. However, this purification does not remove desmethyl sterols, which elute alongside

sterols and hinder their proper quantification. Therefore, different elution conditions are currently being tested to eliminate these interferences. Doing so also the greenness of the methods will be improved. See you soon with more updates! 😊

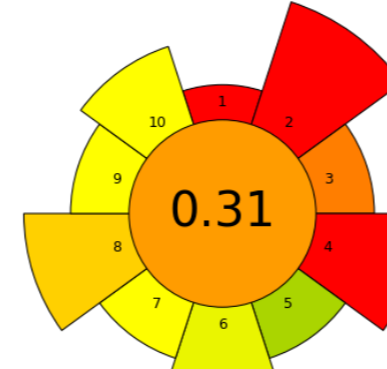
## EVALUATION OF THE GREENNESS



Official  
method COI



MASE+ TLC



MASE+ SPE