

Coupling of Vac-HS-SPME and GC × GC-qMS for simultaneous 5-HMF quantification and volatile profiling in honey

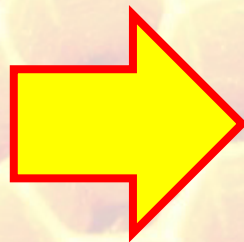
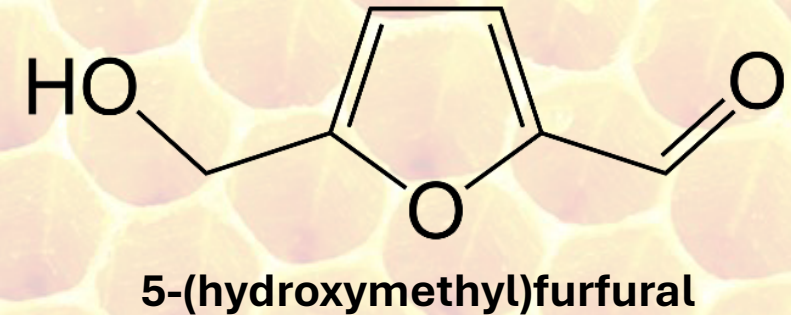
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Why analysing 5-HMF in honey ?

- Heat & storage markers
- Legislated (2001/110/EC):
 - most honey* $\rightarrow \leq 40$ mg/kg
 - from tropical region $\rightarrow \leq 80$ mg/kg
 - Low enzyme activity honey $\rightarrow \leq 15$ mg/kg



LOQ ≤ 15 mg/kg

* Except baker's honey

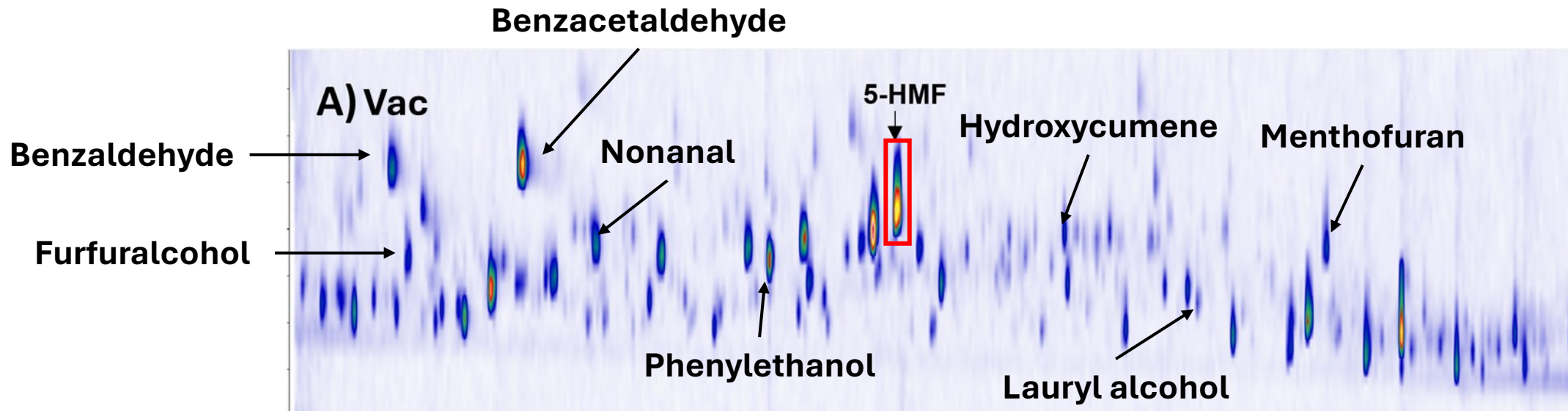


Objectives

- 5-HMF quantification → Targeted → Freshness

Official methods (UV, HPLC-UV)

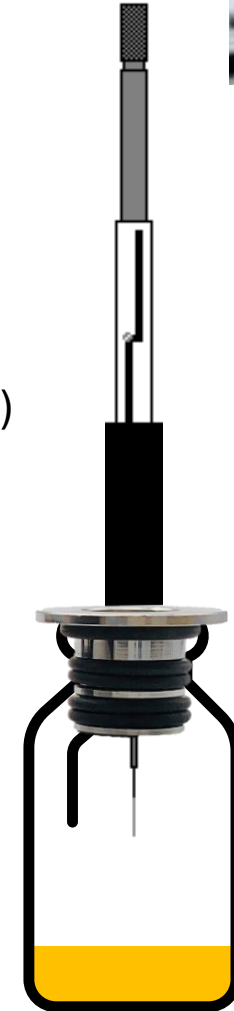
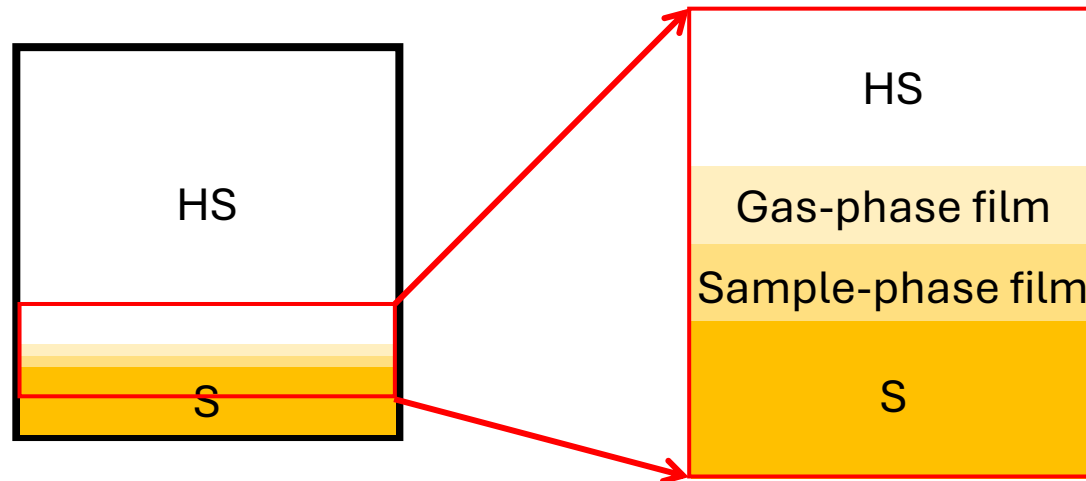
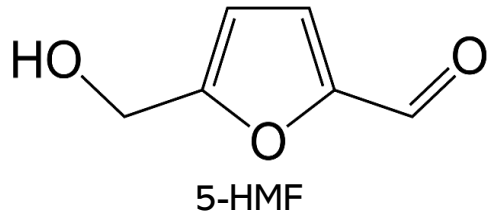
- (Semi-)volatiles → Untargeted → Authenticity, origin, etc.



Vac-HS-SPME

- HS-SPME under vacuum
- Decrease resistance in gas-phase film
- Increase of overall mass-transfer
- Faster equilibration for low/semi-volatile ($K_H < 1.6 \cdot 10^{-4} \text{ atm m}^3 \text{ mol}^{-1}$)

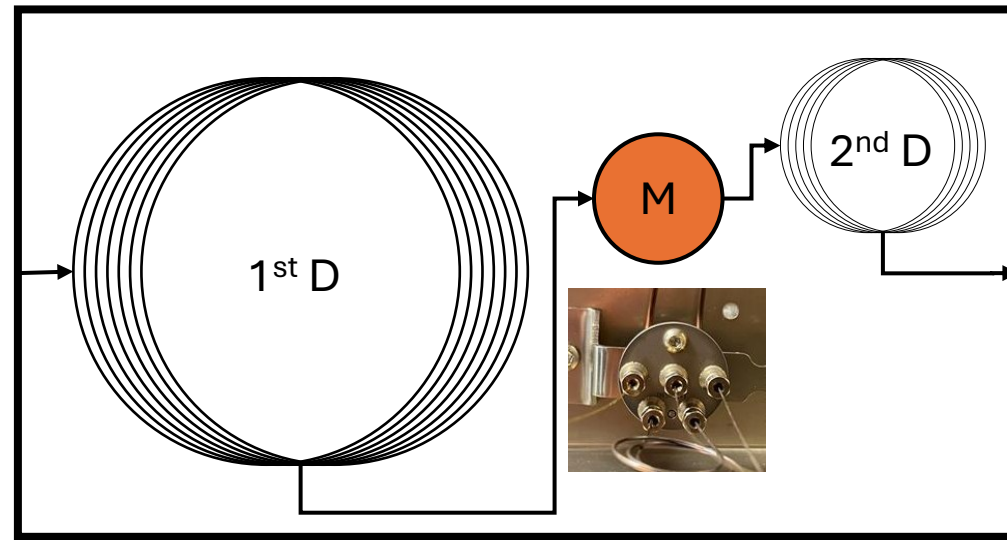
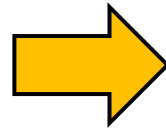
$K_H = 5.41 \cdot 10^{-10} \text{ atm m}^3 \text{ mol}^{-1} \rightarrow$ **Highly beneficial**



Instrumentation



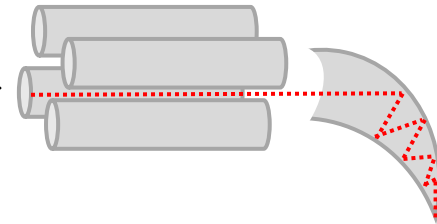
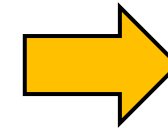
Sample Preparation



Apolar

Polar

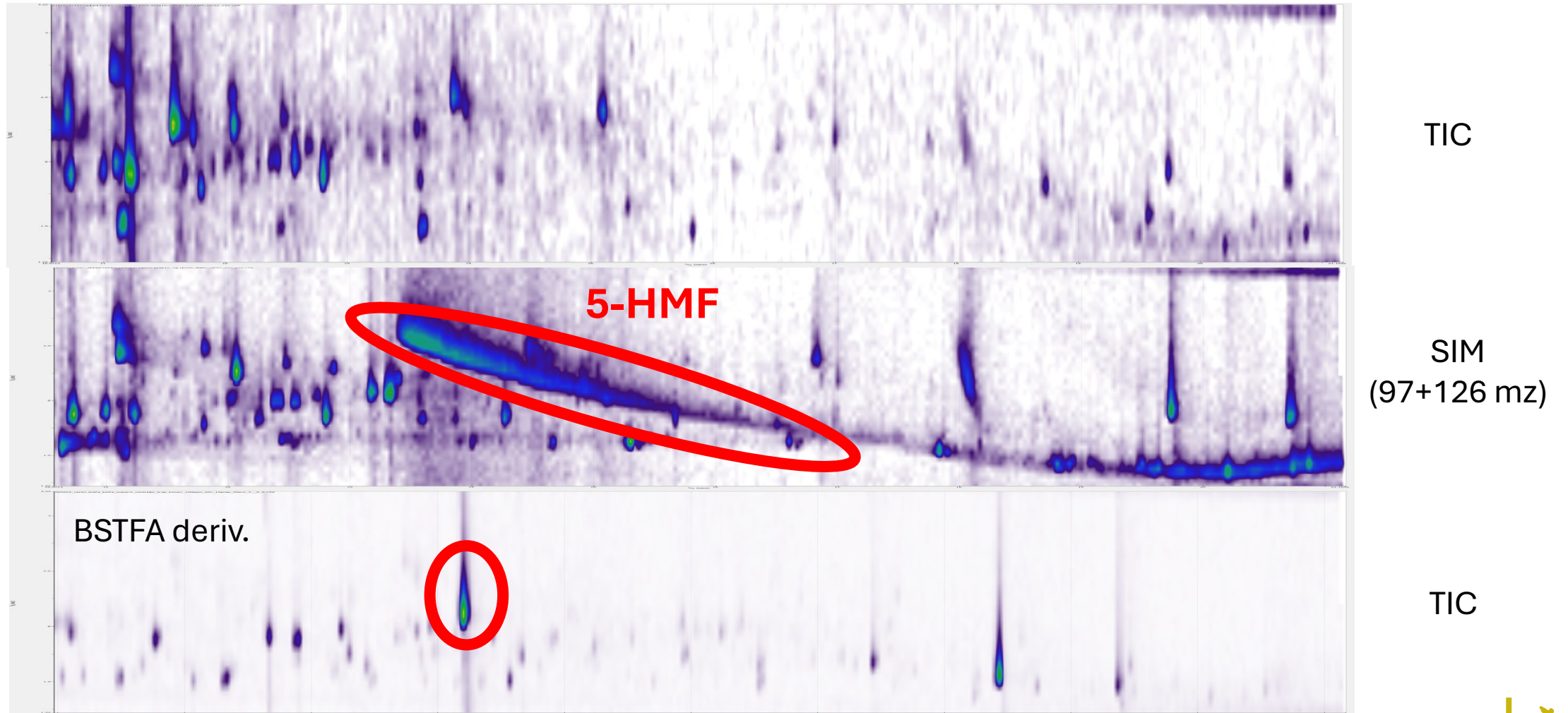
GCxGC



qMS



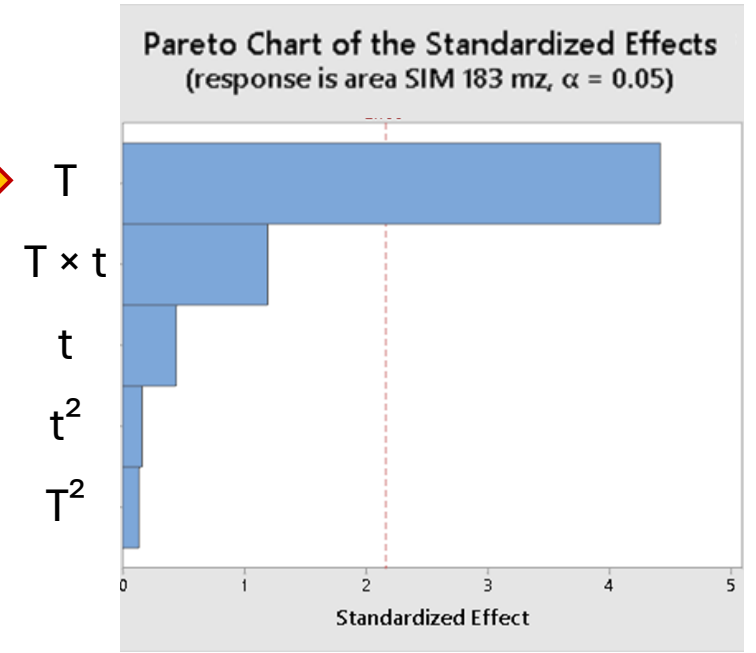
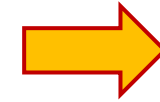
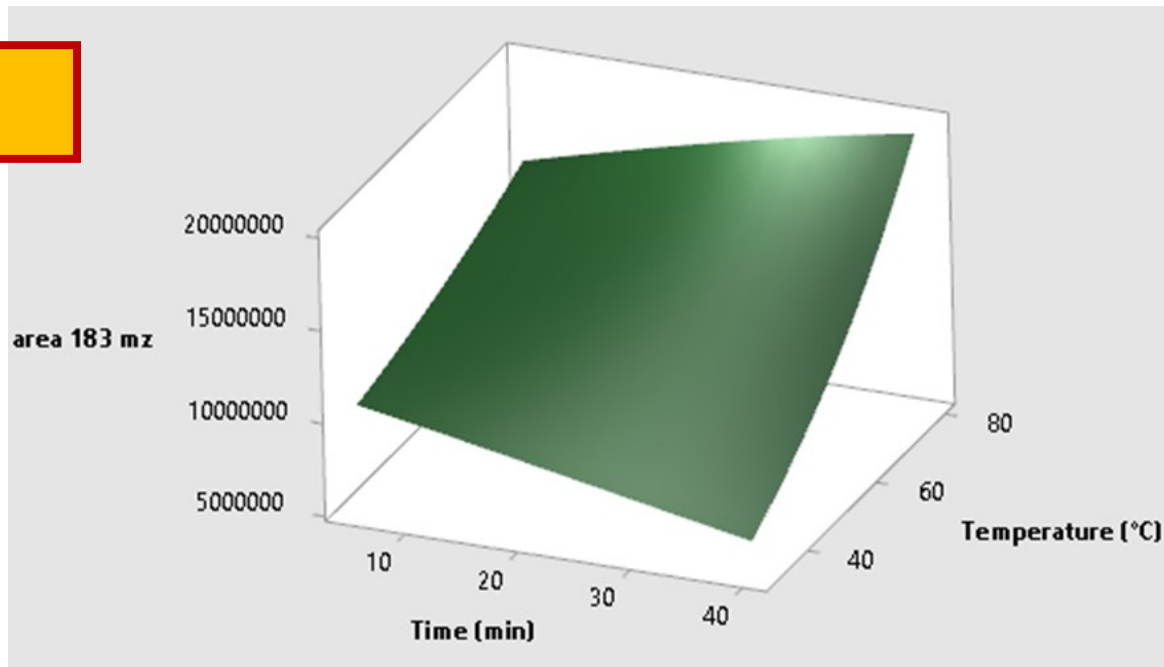
Method development – first trial



Method development – on fiber derivatisation

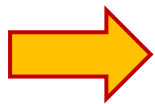
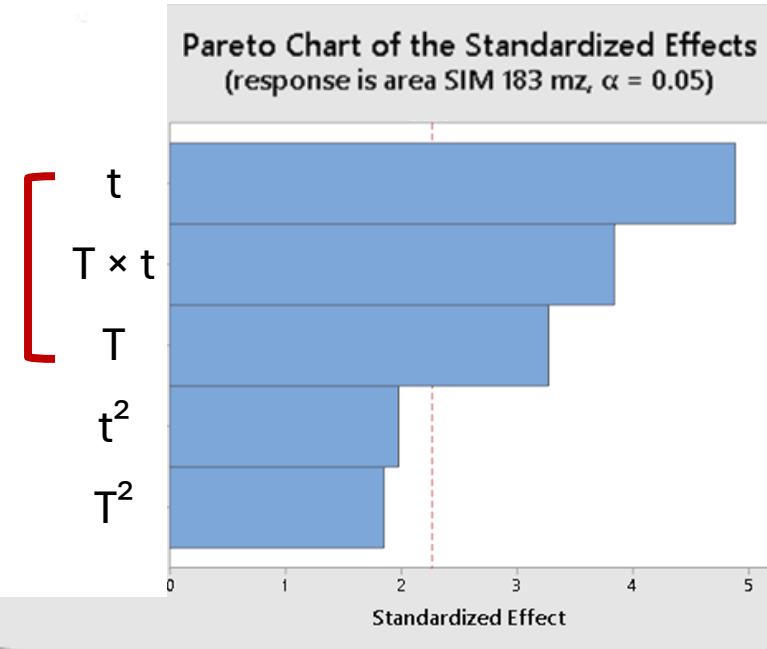
- CCD design
 - Time : 5-40 min
 - Temperature : 30-80 °C
 - Response : 183 mz (SIM) area
 - 50 µg 5-HMF 5 min @ 40°C (20 mL vial)
 - 50 µL BSTFA + 1% TMCS (20 mL vial)

5 min @ 80 °C

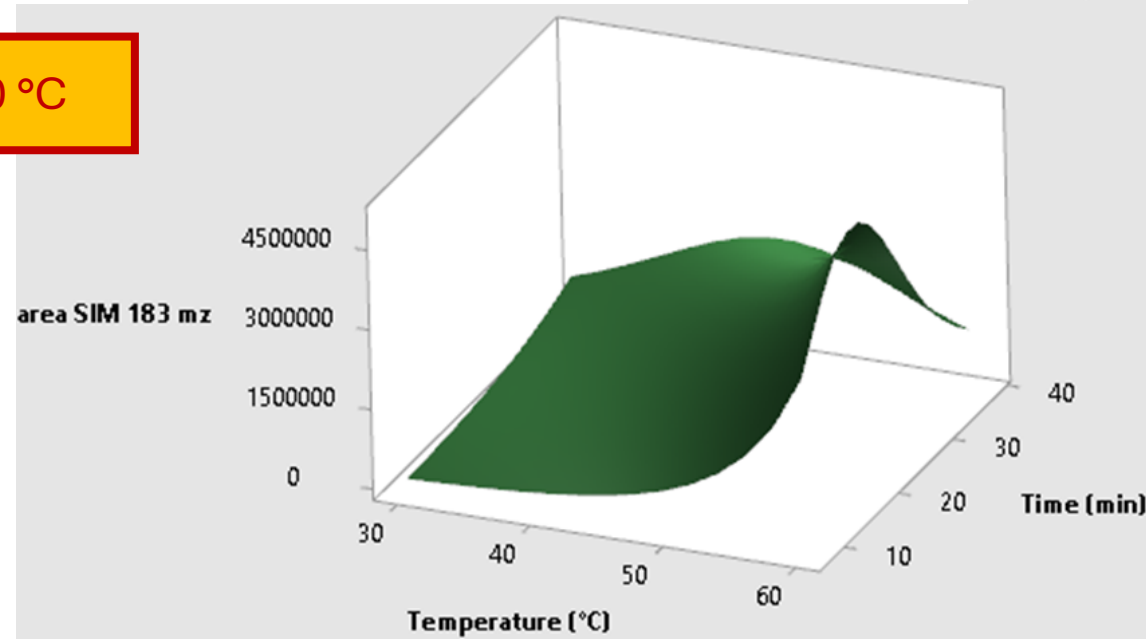


Method development – Extraction

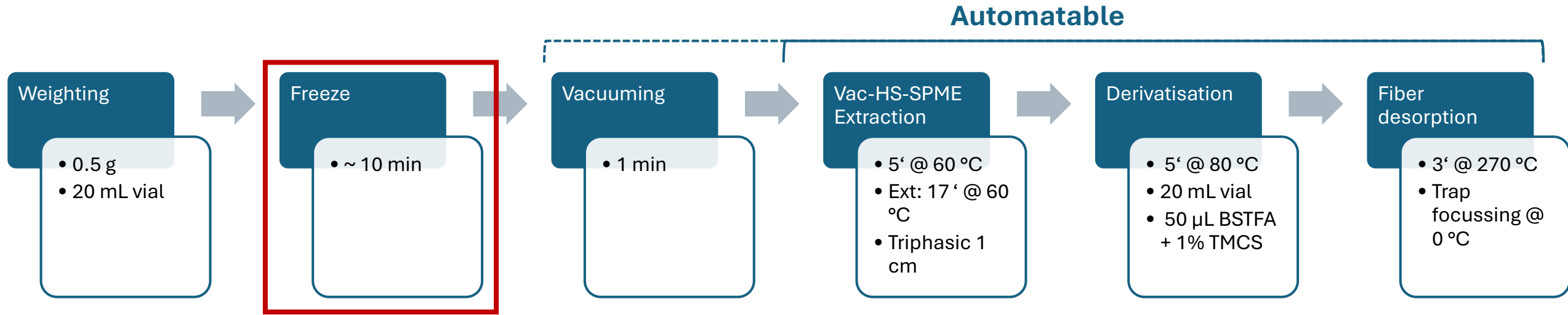
- Doehlert design
 - Time : 5-40 min
 - Temperature : 30-60 °C
 - Response : 183 mz (SIM) area
 - 0.5 g of baked honey (24h @ 60 °C)
 - BSTFA deriv. 5 min @ 80 °C



17 min @ 60 °C

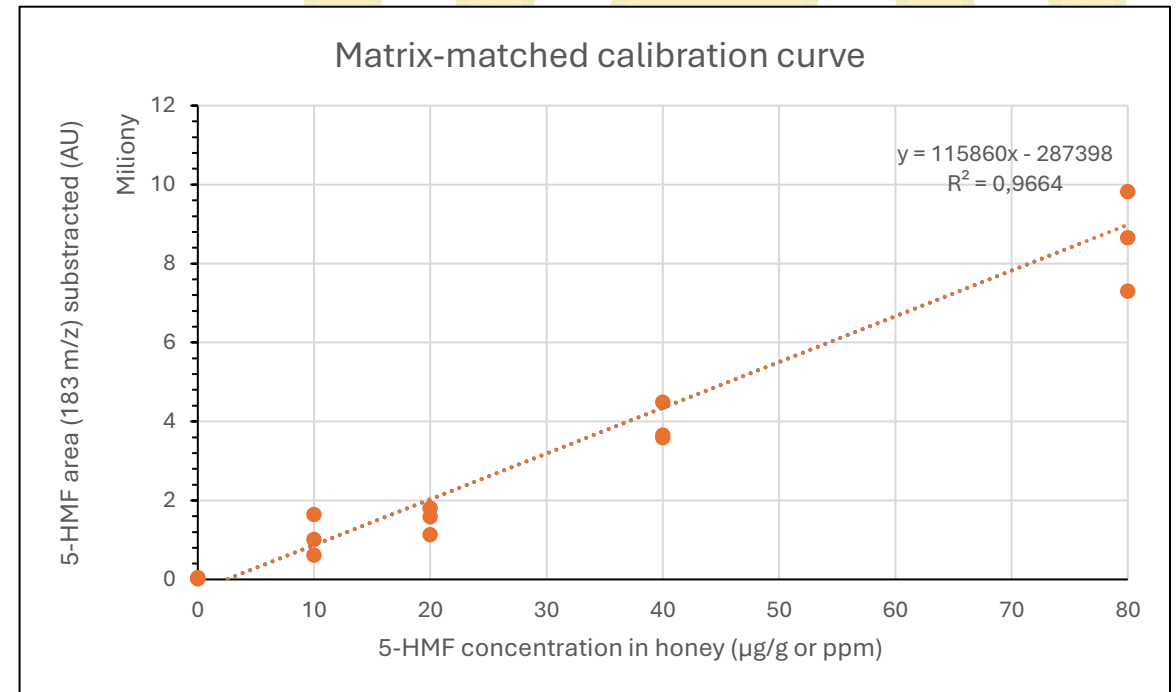


Final method



Method validation

- Matrix-matched calibration at 5 levels (n=3)
- Trueness: avg. recovery of 98 %
- Precision : avg. RSD of 21 %
- LoD : 1.6 $\mu\text{g/g}$
- LoQ : 4.7 $\mu\text{g/g}$ \leq 15 $\mu\text{g/g}$ ✓

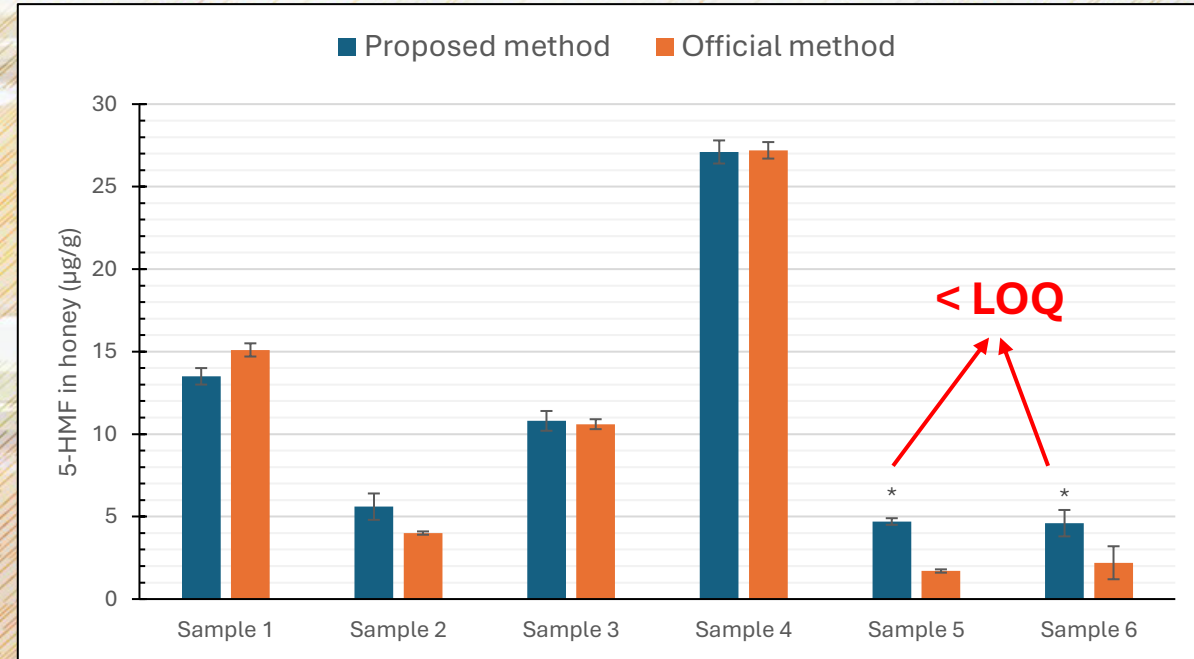


Real-world samples

Vac-SPME-GC×GC-MS method

HPLC-UV method

- 6 diverse samples
- Repeatability : avg. RSD: 8 %
- Bias against HPLC method : 6 %



LOQ : 4.7 µg/g



International Honey Commission

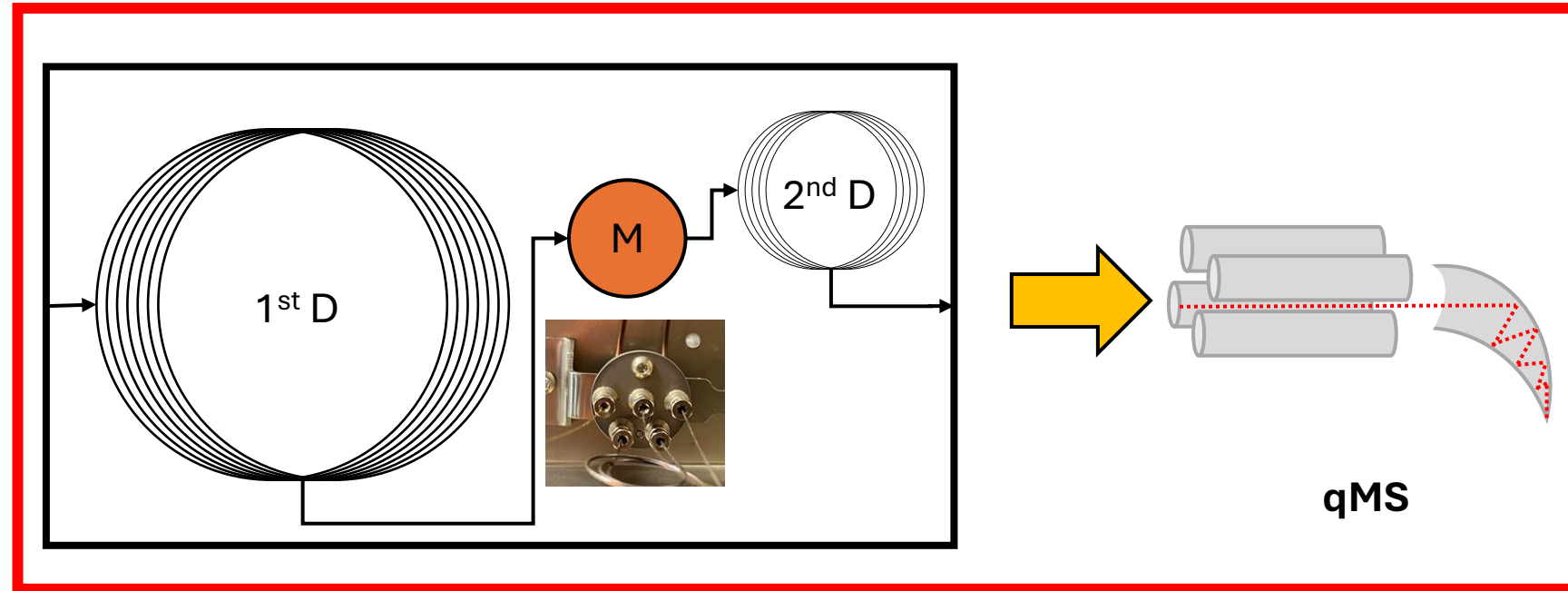
World Network of Honey and Bee Product Science



Instrumentation

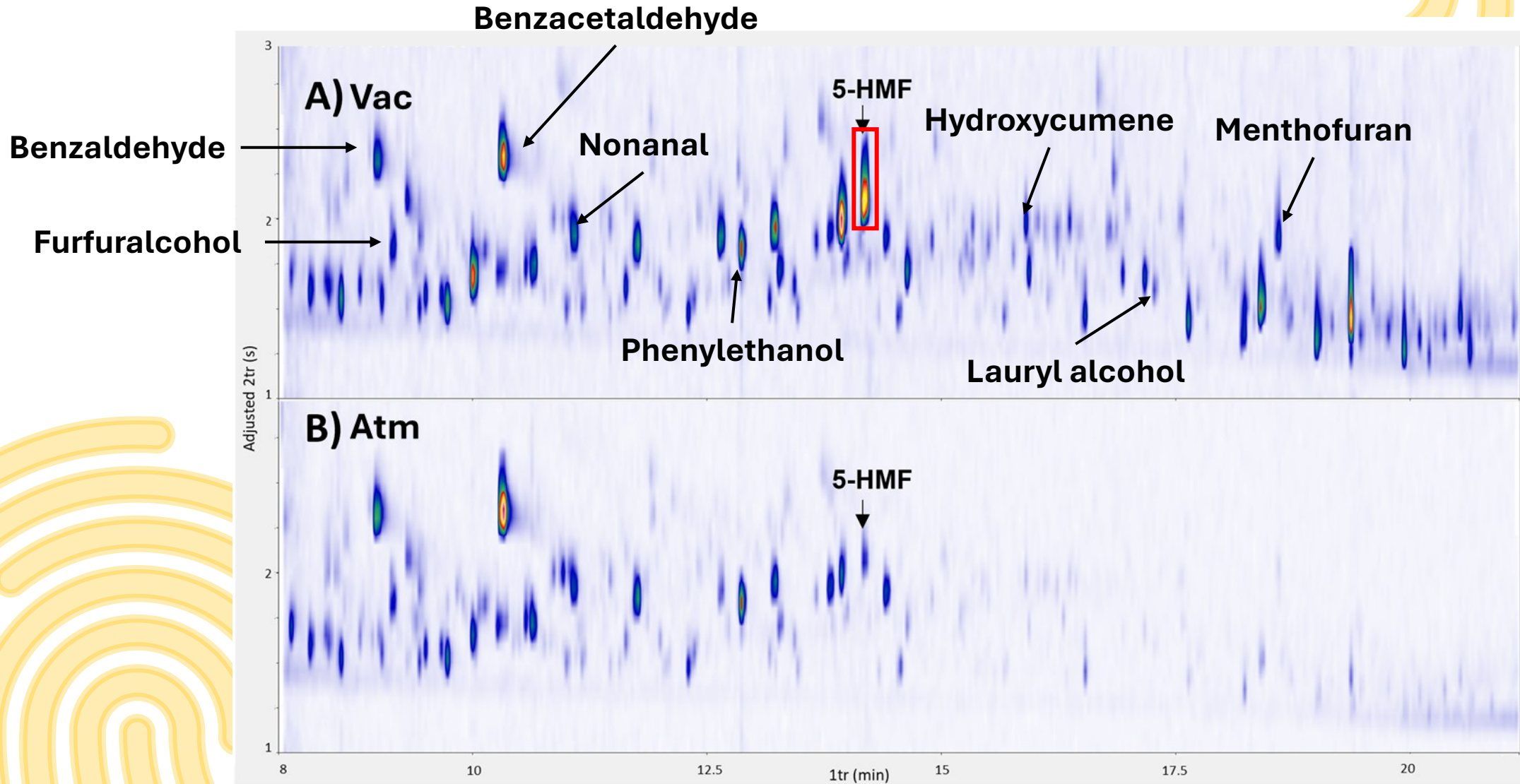


Sample Preparation

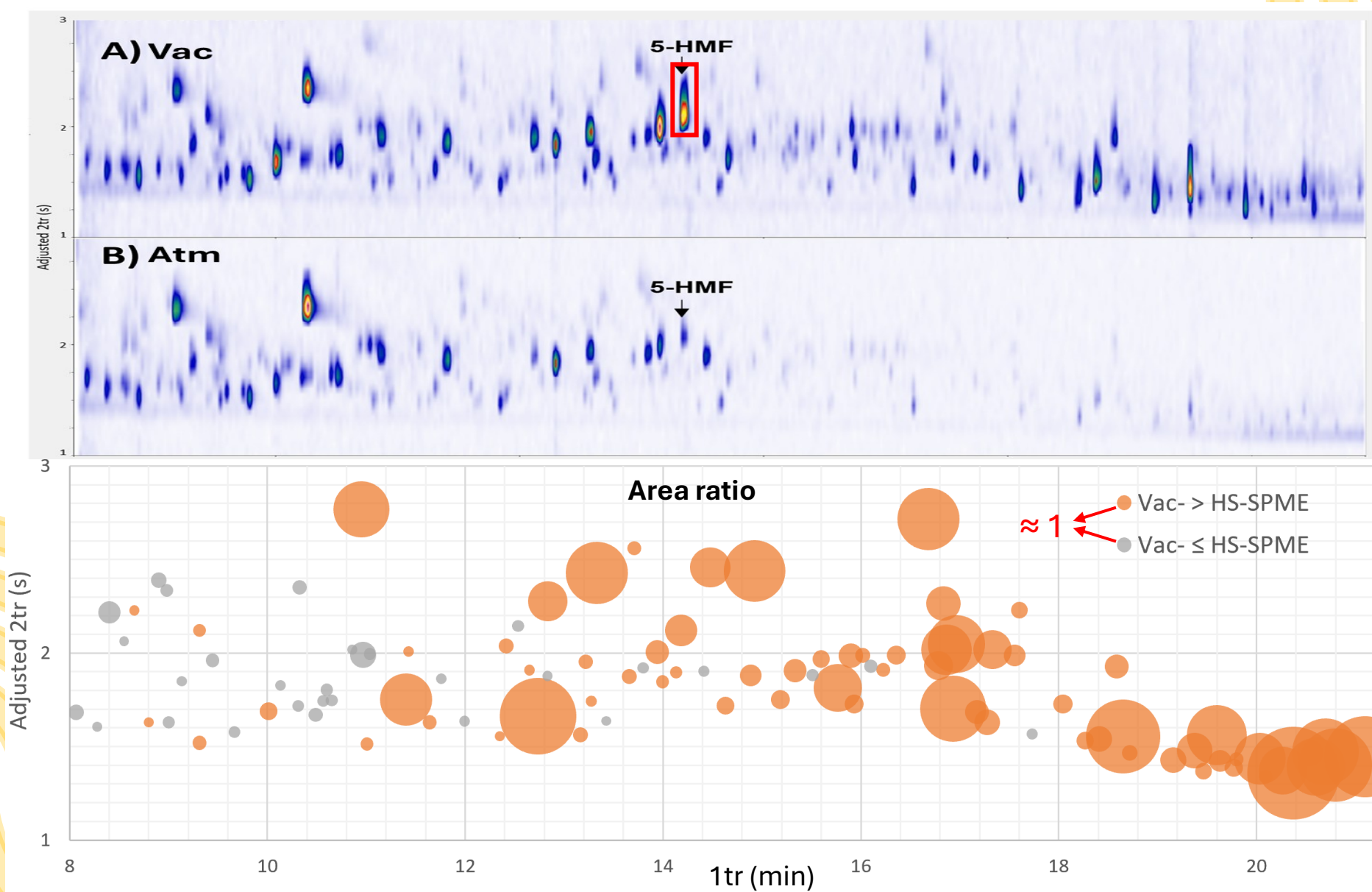


GCxGC

GC×GC for sVOC

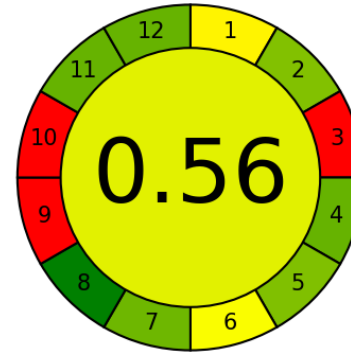


GC×GC for sVOC



Green performances – AGREE

Proposed method



Official method



	<u>Criteria</u>		
2	Sample size	Lower mass sample	
4	Num. of prep. steps		Fewer prep. steps
5	Automation and miniaturisation	Miniaturisation	
6	Derivatisation	50 μ L / 20 samples	No derivatisation
7	Amount of waste	Low waste	
9	Energy consumption		Low energy consumption





Green Analytical Chemistry

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Enhancing the targeted and untargeted analysis of honey by vacuum-assisted SPME-GC × GC-MS. A green, practical, and highly informative approach

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16TH Multidimensional Chromatography Workshop

Thank you for your attention!

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