Estimation of post-mortem interval by LC/HRMS (QTOF) analysis of cuticular lipids in recent and older insect puparia

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Introduction

The purpose of this study is to investigate if an alteration of empty pupae lipidic content is occurring over time. There is also a possible application to estimate the post-mortem interval (PMI).
Preliminary studies conducted by GC/MS have shown that the decomposition of the pupae hydrocarbons appears to depend on time and environmental factors.
Therefore, in order to obtain more information, other compounds such as waxes (up to 60% of the pupae weight) have been studied.
The following trans-esterification reaction was used to observe fatty acid ethyl esters (FAEE) from the waxes (Figure 1), analysed by GC/MS.
Recently, the laboratory has acquired a new LC/HRMS (QTOF). The topic of this study is the identification of wax compounds. Thanks to this analytical technique, new opportunities may arise.

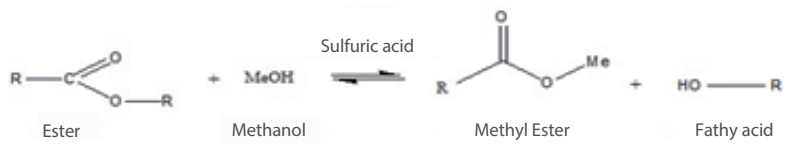

Figure 1: Trans-esterification principle

Experimental procedures

Column: Agilent Poroshell C8 (reverse phase) 2.1 * 100 mm, particle size: 2.7 µm

- Mobile phase: A = Channel ammonium formiate (5 mM) + 0.1% formic acid: buffer solution for the formation of adducts (M+H4)+. B = ACN. D = isopropanol. Ternary gradient elution (Figure 2) pH = 3.1
- Flow rate: 0.5 mL/min
- Temperature: 50°C
- Volume injected: 2 µL
- Infinitely better HPLC 1260, Agilent Technologies
- Ionisation Source: ESI positive Jet Stream Specification Agilent Technologies
- Analyzer: Q-TOF 6540 Accurate-Mass Q-TOF LC\MS, Agilent Technologies
- Mass range: [100-1700] Resolution = 30000

Time [min]	A [%]	B [%]	D [%]
0.00	40.0	30.0	30.0
15.00	5.0	30.0	65.0
25.00	5.0	30.0	65.0

Figure 2: Composition of mobile phase for employed method

Results and discussions

Analysis of saturated esters is possible by LC/HRMS without the trans-esterification reaction.
After pupae are cleaned, extraction with pentane is carried out to obtain fatty esters. Then, they are diluted with isopropanol before being injected to the LC/HRMS. This technique can separate esters (Figure 3).
A database was created with the exact mass and retention times for this analytical method.

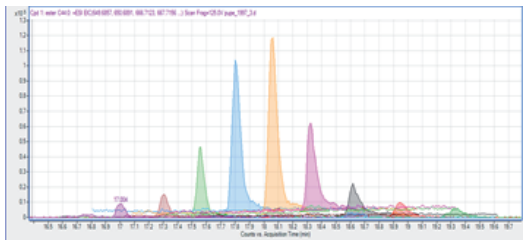

Figure 3: Profile of saturated esters of pupae Hydrotea aenescens, 1997

To investigate the reproducibility of this method, 5 samples of several identical pupae were extracted and injected. For repeatability, a new series was analysed a few weeks later. The results were averaged and corrected.
Thus the importance of the correction of results was highlighted. Each saturated ester was divided by the sum of the areas of all the esters from the pupae. Moreover, the results of each ester were corrected on 5 pupae series (Figure 4).

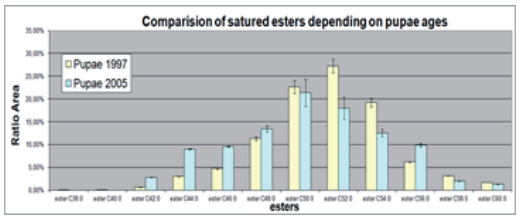

Figure 4: Area comparison depending on the pupae age

After correction, the results of the qualitative analysis suggest the possibility of estimating the post-mortem period. Indeed, variations in the ratios of esters were found between pupae of two different ages.
The analyses by LC/MS² have to achieve and it will allow identification of the composition of the two aliphatic chains without distinguishing their ramifications.

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