

# HS-SPME-GC-MS Analyses of Volatiles in Plant Populations – Quantitating Compound × Individual Matrix Effects

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## SUPPORTING INFORMATION

### MATERIALS & METHODS

**Chemical Reagents and Standards for [U<sup>13</sup>C]Hexanal and [U<sup>13</sup>C]Hexanol Synthesis.** The following chemicals were purchased from Sigma-Aldrich (St. Louis, MO): linoleic acid (95%), [U<sup>13</sup>C]  $\alpha$ -linoleic acid (>97%; >98% <sup>13</sup>C enrichment), Soybean lipoxygenase (LOX) (EC No. 1.13.11.12) type I-B (221700 units/mg), alcohol dehydrogenase (ADH) from *Saccharomyces cerevisiae* (15000 units/mg),  $\beta$ -Nicotinamide adenine dinucleotide (NADH), reduced disodium salt hydrate (>94%), hexanal ( $\geq$ 97%), and hexanol ( $\geq$ 98%). Chemicals for buffers (citric acid ( $\geq$ 99%), sodium phosphate mono- ( $\geq$ 99%), and di-basic ( $\geq$ 98%), sodium bicarbonate ( $\geq$ 99%), and sodium carbonate ( $\geq$ 99%)) and organic solvents – ethanol ( $\geq$ 98%; EtOH), methanol ( $\geq$ 99%; MeOH), dichloromethane ( $\geq$ 99%; DCM), and pentane ( $\geq$ 99%) – were also purchased from Sigma Aldrich.

### Preparation of Stock and Working Solutions for [U<sup>13</sup>C]Hexanal and [U<sup>13</sup>C]Hexanol

#### Synthesis

Enzyme solutions: Separate solutions of LOX (753780 units/mL) and ADH (39300 units/mL) stock solution were prepared by addition to 20 mL of Milli-Q water. Each stock was then stored in glass vials at -80 °C in 1.5 mL aliquots and thawed prior to use.

Chemical Standards: A solution of linoleic acid (5% w/w) was prepared by weighing 0.5 g of linoleic acid into 9.5 g of EtOH. [U<sup>13</sup>C]linoleic acid stock solution (0.1 g was diluted in EtOH solution yielding a 5.95% w/w stock solution. Unlabeled hexanal and hexanol were prepared in EtOH to yield 10 and 100  $\mu$ g/mL working solutions. NADH stock solution was prepared by adding 20 mL of Milli-Q water into 1 g of NADH yielding a stock concentration of 0.05 g/mL.

Buffer solutions: pH 4.5, pH 7.0, and pH 9.5 buffer solutions were prepared from 0.1 M citric acid/0.2 M sodium phosphate dibasic, 0.1 M sodium phosphate dibasic /0.1 M sodium phosphate monobasic, and 0.1 M sodium bicarbonate/0.1 M sodium carbonate respectively. The solutions were stored at 3 °C.

### Protocol for Enzymatic Synthesis of [U-<sup>13</sup>C]hexanal and [U-<sup>13</sup>C]hexanol from [U-<sup>13</sup>C]α-linoleic acid

The protocol for generating [U-<sup>13</sup>C]hexanal and [U-<sup>13</sup>C]hexanol is shown in Figure S.1. The yield of hexanal and hexanol was determined by calibration against unlabeled standards on GC-MS.

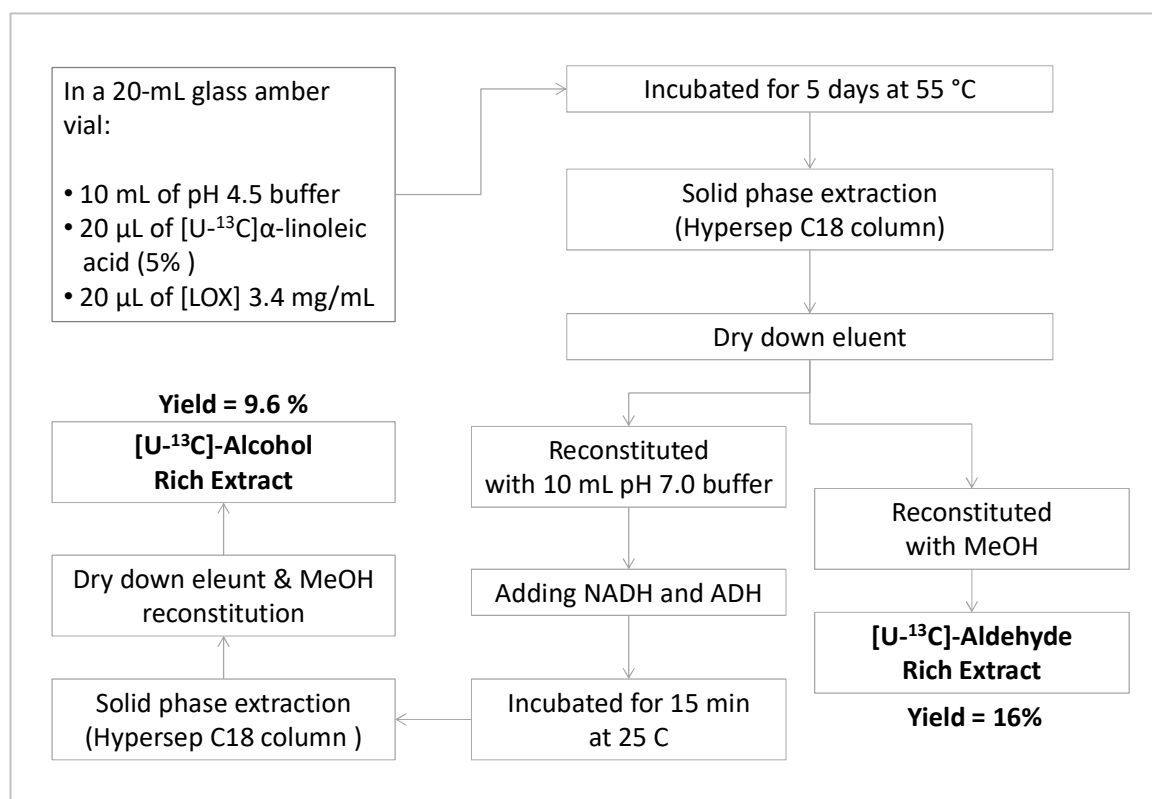


Figure S.1 – Protocol for generation of [U-<sup>13</sup>C]hexanal and [U-<sup>13</sup>C]hexanol from [U-<sup>13</sup>C]α-linoleic acid.

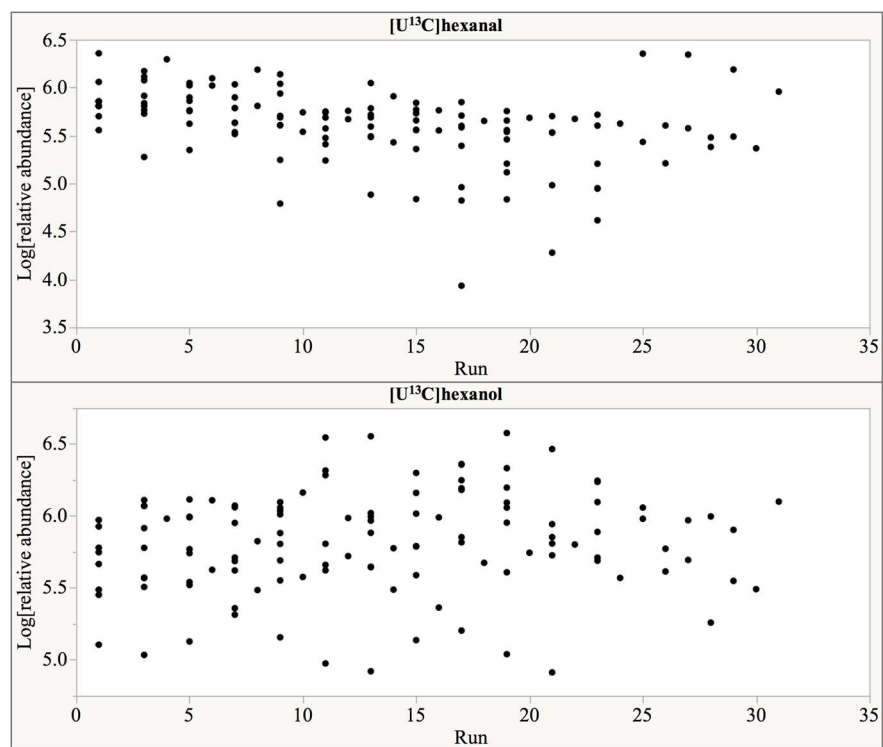


Figure S.2: Plot of ordinal run number (i.e. sample queue assignment) versus log-normalized peak areas for [U<sup>13</sup>C]hexanal (top) and [U<sup>13</sup>C]hexanol (bottom).