# Short chain fatty acid analysis

## Analysis summary

The assay is intended to generate the profile of short chain (C2-C7) fatty acids in several types of biological matrix. Analysis is performed by gas chromatography – mass spectroscopy. Absolute quantitation (concentrations of analytes in µM) is obtained using appropriate internal standards, data are normalized to original sample weight or volume. Assay coefficient of variation is usually within 10%.

## Preparation of isotopically labeled internal standards (IS)

The standards used are listed in table 1 below. The working solution is prepared by mixing of 1470µL of water with 15µL of 50mM Hexanoic-d11 acid and 15µL of 400mM Butyric-d7 acid**.**

Table . SCFA isotope-labeled internal standards solution stock (IS)

|  |  |  |
| --- | --- | --- |
| Compound | Supplier | Catalog #  |
| Hexanoic-d11 acid | Aldrich(?) | 448168 |
| Butyric-d7 acid | Aldrich(?) | 488399 |

## Preparation of unlabeled standard mixture (STD mix)

Mixture of authentic standards (STD mix) is prepared by adding stock solutions specified in table 2 below to 700µL of water (LCMS-grade or Milli-Q) to yield 1ml of working solution.

Table . SCFA internal standards solution stock (STD mix)

| Compound | Stock concentration | Volume  |
| --- | --- | --- |
| acetic acid | 400mM | 37.5µL |
| propionic acid | 400mM | 37.5µL |
| n-butyric acid | 400mM | 37.5µL |
| iso-butyric acid | 50mM | 37.5µL |
| n-valeric acid | 200mM | 37.5µL |
| iso-valeric acid | 200mM | 37.5µL |
| n-caproic acid | 50mM | 37.5µL |
| n-heptanoic acid | 15mM | 37.5µL |

## Preparation of calibration standards

Calibration standards are prepared in glass auto-sampler vials according to table 3 below.

Table . SCFA internal standards calibration curve\*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Standard | Concentration, µM | STD mix (µL) | IS (µL) | Water (µL) | 1M HCl (µL) |
| STD 0 | 0 | 0 | 18.75 | 272 | 9 |
| STD 1 | 0.1 | 2 | 18.75 | 270 | 9 |
| STD 2 | 0.3 | 6 | 18.75 | 266 | 9 |
| STD 3 | 1 | 20 | 18.75 | 252 | 9 |
| STD 4 | 3 | 60 | 18.75 | 212 | 9 |
| STD 5 | 10 | 200 | 18.75 | 72 | 9 |

\*The concentrations of standards may vary from experiment to experiment depending on the nature of the samples and range of concentrations that has to be covered by the assay.

## Analyte extraction and sample preparation

### Fecal matter extraction

* Extraction solvent
	+ 6.25% v/v isotopically labeled internal standards (IS) stock
	+ 3% v/v 1M HCl
	+ 90.75% v/v water
* Remove the samples from -80°C and place them on ice for the whole duration of the extraction procedure.
* Add 1 scoop of 0.5mm and 1mm zirconium beads to each pre-labeled 1.5mL “safe lock” micro-centrifuge tube or Rhino tube, weigh the tubes with beads and record the weights.
* Add the sample (~50 - 100mg) and re-weight test tubes to determine the exact amount of biological material.
* Add 600µL of cold extraction solvent and homogenize in a bead-beater at -20°C, power level 8 for 3min; alternatively homogenization may be performed by sonication without using the beads.
* Vortex samples for 10sec, keep on ice or at -20oC for 10min, vortex again.
* Transfer 300µL of suspension to a glass tube, centrifuge for 10min at 4°C, 4000rpm.
* Transfer the supernatant to glass auto-sampler vials, set pellets aside for dry weight determination later.
* Create pooled sample by combining 10uL aliquots from each individual sample.
* Add 300µL of diethyl ether to sample extracts and calibration standards, vortex for 10sec to emulsify, leave for 5min on ice to allow initial phase separation.
* Centrifuge for 1min at 4°C, 15,000rpm; if upper layer remains cloudy add more diethyl ether, vortex and centrifuge again.
* Transfer upper layer to pre-labeled auto-sampler vial with insert and immediately cap (use glued caps from Phenomenex or analogous to prevent evaporation).
* Promptly analyze by GC-MS.
* Vacuum-dry the pellets obtained after first extraction at 45oC and determine their weights for subsequent data normalization.

### Blood plasma, serum or bronchoalveolar lavage fluid (BALF) extraction

* Extraction solvent
	+ 3.35% v/v isotopically labeled internal standards (IS) stock
	+ 1.25% v/v 12M HCl
	+ 95.4% v/v water
* Remove the samples from -80°C and place them on ice for the whole duration of the extraction procedure, allow to thaw.
* Transfer 100 µL of sample to a glass tube (12x75mm).
* Add 200 µL of extraction solvent, mix well, centrifuge if cloudy.
* Create pooled sample by combining 10uL aliquots from each individual sample.
* Vortex the samples, add 300µL of diethyl ether to sample extracts and calibration standards, vortex for 10sec to emulsify, leave for 5min on ice to allow initial phase separation.
* Centrifuge for 1min at 4oC, 4000rpm to separate layers, transfer upper layer to auto-sampler vial with insert, cap vials immediately.
* Promptly analyze by GC-MS.

## GC-MS

Samples are analyzed on ZB-WAX Plus 30x0.25x0.25 column from Phenomenex. The specific GC-MS method details are provided in WAXPLUS\_100-200C\_2\_SIM.txt file.

## References