# Bile acid analysis

## Analysis summary

The assay is intended to generate the profile of thirty species of bile acids in various biological matrices. Analysis is performed by liquid chromatography – mass spectroscopy. Absolute quantitation (µM concentrations of analytes) is obtained using appropriate internal standards, data are normalized to original sample weight/volume. Assay coefficient of variation is usually within 5%.

## Bile acids internal standards preparation

#### Unlabeled bile acids mixture (BA mix)

Two methanol stock solutions are prepared containing the bile acids listed in table 1 below. One stock contains each standard at 1 µM (BA1) and another at 10µM (BA10) final concentration

Table 1. Unlabeled bile acid standards

|  |  |
| --- | --- |
| Name | Abbreviation |
| Glycodeoxycholate | GDCA |
| Glycochenodeoxycholate | GCDCA |
| Glycolithocholate | GLCA |
| Taurodeoxycholate | TDCA |
| Taurochenodeoxycholate | TCDCA |
| Hyodeoxycholate | HDCA |
| α-Muricholate | aMCA |
| β-Muricholate | bMCA |
| ω-Muricholate | wMCA |
| γ-Muricholate (hyocholate) | HCA |
| Glycoursodeoxycholate | GUDCA |
| Tauroursodeoxycholate | TUDCA |
| Tauro-hyodeoxycholate | THDCA |
| Glycol-hyodeoxycholate | GHDCA |

#### Isotope-labeled bile acids internal standards stock (IS)

Methanol stock solution containing each of the standards listed in table 2 below at 100µM final concentration is diluted 1:10 (10µM of each compound)

Table 2. Isotope labeled bile acids

|  |  |
| --- | --- |
| Name | Abbreviation |
| d4-Cholate | d4-CA |
| d4-Lithocholate | d4-LCA |
| d4-Glycocholate | d4-GCA |
| d4-Glycochenodeoxycholate | d4-GCDCA |
| d4-Deoxycholate | d4-DCA |

#### Bile acids calibration solutions

Calibration standards for bile acid quantitation are prepared according to table 3 below.

Table 3. Bile acids calibration standards

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Standard name | Concentration, µM | Ethanol, µL | Chloroform, µL | Methanol, µL | BA1, µL | BA10, µL | IS, µL |
| STD 0 | 0 | 250 | 125 | 115 |  |  | 5 |
| STD 1 | 10 | 250 | 125 | 110 | 5 |  | 5 |
| STD 2 | 50 | 250 | 125 | 90 | 25 |  | 5 |
| STD 3 | 100 | 250 | 125 | 111 |  | 5 | 5 |
| STD 4 | 500 | 250 | 125 | 95 |  | 25 | 5 |
| STD 5 | 2000 | 250 | 125 | 15 |  | 100 | 5 |

## Analyte extraction and sample preparation

Extraction solvent A – 100% Ethanol containing 0.1µM IS.

Extraction solvent B – chloroform : methanol 1 : 1 containing 0.1µM IS.

### Extraction procedure

* Keep the samples on ice throughout the whole procedure.
* Transfer ~50 - 100 mg of the sample to pre-weighted, pre-labeled micro-centrifuge tube, weight the tube to determine exact amount of the sample for normalization.
* Add 1mL of chilled Solvent A.
* Sonicate the sample at 40% output power, 20% duty cycle for 5 seconds using the probe sonicator; keep test tube submerged in ice during sonication.
* Vortex for 10sec, leave on ice or at -20oC for 10 min, vortex again.
* Transfer 250µL of sample suspension to pre-labeled glass test tube (12x75mm).
* Centrifuge remaining sample at for 10min at 4°C, 14,000rpm.
* Transfer supernatant to a pre-labeled glass auto-sampler vial (extract A).
* Add 250µL of Solvent B to sample suspension in the glass test tube, vortex.
* Incubate on ice for 10min, vortex again.
* Centrifuge for 1min at 4°C, at 4000rpm combine upper layer with extract A for the corresponding sample.
* Dry the samples and calibration standards at 45°C in vacuum centrifuge (45min to 1 hour).
* Reconstitute the samples with 120µL of 50% methanol in water.
* Create pooled sample by combining 10µL aliquots of each individual sample.
* Transfer reconstituted samples to auto-sampler vials with glass inserts, centrifuge the vials for 1min at 4°C, at 4000rpm.

## LC-MS

* Chromatographic column - ACQUITY UPLC BEH C18, 130Å, 1.7 µm, 2.1mm X 50mm (Waters).
* LC gradient
  + Phase A: acetonitrile : methanol 3 : 1 with 10mM ammonia acetate.
  + Phase B: 10mM ammonia acetate in water, pH 8.0
  + timetable – listed in table 4 below
* Auto-sampler temperature 4°C.
* Injection volume 10µL.
* Mass-spectrometer parameters
  + Instrument - Agilent 6410 QQQ
  + Mode – ESI negative.
* Monitored MRM transitions for individual ceramides are listed in table 5 below.

The specific LC-MS method details are provided in supplementary material (2014-07-30-Bile\_acid-full-BEH-C18-I\_LC\_PARAMS.xml and 2014-07-30-Bile\_acid-full-BEH-C18-I\_MS\_PARAMS.xml files).

Table 4 LC gradient timetable

|  |  |  |
| --- | --- | --- |
| Time, min | %B | Flow, ml/min |
| 0 | 5 | 0.27 |
| 0.5 | 5 | 0.27 |
| 3 | 25 | 0.27 |
| 17 | 40 | 0.27 |
| 19 | 95 | 0.27 |
| 21 | 95 | 0.27 |
| 21.1 | 5 | 0.27 |
| 24 | 5 | 0.27 |

Table 5 MRM transitions for individual bile acids

| Name | MS1 M/Z | MS2 M/Z | Dwell time | Fragmentor, V | Collision energy, V | Polarity |
| --- | --- | --- | --- | --- | --- | --- |
| Taurocholate | 514.3 | 80 | 50 | 380 | 35 | Negative |
| α-tauro-muricholate | 514.2 | 80 | 50 | 380 | 70 | Negative |
| β-tauro-muricholate | 514.2 | 80 | 50 | 380 | 70 | Negative |
| γ-tauro-muricholate | 514.2 | 80 | 50 | 380 | 70 | Negative |
| Taurohyodeoxycholate | 498.3 | 80 | 50 | 380 | 70 | Negative |
| Tauroursodeoxycholate | 498.3 | 80 | 50 | 380 | 70 | Negative |
| Taurodeoxycholate | 498.3 | 80 | 50 | 380 | 70 | Negative |
| Taurochenodeoxycholate | 498.3 | 80 | 50 | 380 | 70 | Negative |
| Taurolithocholate | 482.3 | 80 | 50 | 380 | 70 | Negative |
| d4-Glycocholate | 468.3 | 74 | 50 | 380 | 13 | Negative |
| Glycocholate | 464.3 | 74 | 50 | 380 | 13 | Negative |
| d4-Glycochenodeoxycholate | 452.3 | 74 | 50 | 380 | 13 | Negative |
| Glycohyodeoxycholate | 448.3 | 74 | 50 | 380 | 13 | Negative |
| Glycoursodeoxycholate | 448.3 | 74 | 50 | 380 | 13 | Negative |
| Glycodeoxycholate | 448.3 | 74 | 50 | 380 | 13 | Negative |
| Glycochenodeoxycholate | 448.3 | 74 | 50 | 380 | 13 | Negative |
| Glycolithocholate | 432.3 | 74 | 50 | 380 | 13 | Negative |
| d4-Cholate | 411.3 | 411.3 | 50 | 380 | 3 | Negative |
| Hyocholate | 407.3 | 407.3 | 50 | 380 | 3 | Negative |
| ω-Muricholate | 407.3 | 407.3 | 50 | 380 | 3 | Negative |
| β-Muricholate | 407.3 | 407.3 | 50 | 380 | 3 | Negative |
| α-Muricholate | 407.3 | 407.3 | 50 | 380 | 3 | Negative |
| Cholate | 407.3 | 407.3 | 50 | 380 | 3 | Negative |
| d4-Deoxycholate | 395.3 | 395.3 | 50 | 380 | 3 | Negative |
| Hyodeoxycholate | 391.3 | 391.3 | 50 | 380 | 3 | Negative |
| Ursodeoxycholate | 391.3 | 391.3 | 50 | 380 | 3 | Negative |
| Deoxycholate | 391.3 | 391.3 | 50 | 380 | 3 | Negative |
| Chenodeoxycholate | 391.3 | 391.3 | 50 | 380 | 3 | Negative |
| d4-Lithocholate | 379.3 | 379.3 | 50 | 380 | 3 | Negative |
| Lithocholate | 375.3 | 375.3 | 50 | 380 | 3 | Negative |

## References

William J. Griffiths and Jan Sjövall, Bile acids: analysis in biological fluids and tissues, *Journal of Lipid Research*, **2010**, 51, 23