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Data Article

Data supporting development and validation of liquid chromatography tandem mass spectrometry method for the quantitative determination of bile acids in feces

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A B S T R A C T

Measuring bile acids in feces has an important role in disease prevention, diagnosis, treatment, and can be considered a measure of health status. Therefore, the primary aim was to develop a sensitive, robust, and high throughput liquid chromatography tandem mass spectrometry method with minimal sample preparation for quantitative determination of bile acids in human feces applicable to large cohorts. Due to the chemical diversity of bile acids, their wide concentration range in feces, and the complexity of feces itself, developing a sensitive and selective analytical method for bile acids is challenging. A simple extraction method using methanol suitable for subsequent quantification by liquid chromatography tandem mass spectrometry has been reported in, “Extraction and quantitative determination of bile acids in feces” [1]. The data highlight the importance of optimization of the extraction procedure and the stability of the bile acids.

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Specifications Table

| Subject                          | Analytical Chemistry                      |
|---------------------------------|-------------------------------------------|
| Specific subject area           | Analytical, bioanalytical, and clinical chemistry, Liquid Chromatography Mass Spectrometry (LC-MS/MS) analysis |
| Type of data                    | Excel spreadsheet                          |
| How data were acquired          | An Ultimate 3000 Liquid Chromatography coupled to a TSQ Quantiva Triple Quadrupole Mass Spectrometer from Thermo Scientific (CA, USA). |
| Data format                     | Raw data: Microsoft Excel                  |
| Parameters for data collection  | Analysed output data: Microsoft Excel      |
| Description of data collection  | Fecal samples were collected from all bowel motions over a 24 h period from healthy adult individuals. If more than one stool sample was collected, they were homogenised as individual samples and then pooled and homogenised again. A quantitative approach for the determination of bile acids in feces samples using LC-MS/MS was developed and extraction methods were optimized. The raw data were acquired with the use of Xcalibur 4.1 software (Thermo Scientific, CA, USA) and data processing was performed with Trace Finder™ 4.1 software (Thermo Scientific, CA, USA). |
| Data source location            | Institution: centre for Integrative Metabolomics and Computational Biology, School of Science, Edith Cowan University |
|                                 | City/Region: Joondalup, Western Australia |
|                                 | Country: Australia                         |
|                                 | Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: 31°45′09.5″S115°46′30.2″E |
| Data accessibility              | Data identification number: DOI:10.17632/fvy6cz8bgj.1 |
|                                 | DirectURL to data: https://data.mendeley.com/datasets/fvy6cz8bgj.1 |
| Related research article        | A. Shafaei, J. Rees, C.T. Christophersen, A. Devine, D. Broadhurst, M.C. Boyce, Extraction and quantitative determination of bile acids in feces, Anal. Chim. Acta. 1150 (2021) 338,224. |
|                                 | https://doi.org/10.1016/j.aca.2021.338224 |

Value of the Data

- The data highlight challenges for the development of a robust, selective, and sensitive analytical method for quantitative determination of bile acids in complex feces matrix.
- The optimized extraction method can be used in future LC-MS/MS method development for quantification of bile acids in other biological samples such as urine and serum.
- The data can be used by other scientists as a workflow for development of targeted metabolomics assay in feces.

1. Data Description

Wet vs dry samples.xlsx dataset.

This Excel workbook includes the full raw and analysed data obtained from dried feces (D); dried feces spiked with deuterated internal standards prior to drying (SD); and wet feces (W) extracts for each bile acid.
The data for each bile acid is provided in a separate sheet.

Stability_RT vs 6C.xlsx dataset.

This Excel workbook includes the full raw and analysed data obtained from bile acid standard solutions (SS1-SS5) and bile acids in feces (Ext1-Ext4) after 12 and 24 h storage at room temperature and at 6 °C.

The data for each bile acid is provided in a separate sheet.

Stability_freeze_thaw_cycles.xlsx dataset.

This Excel workbook provides the full raw and analysed data for bile acid standard solutions (SS1-SS5) and bile acids in feces (Ext1-Ext4) after multiple freeze thaw cycles.

The data for each bile acid is provided in a separate sheet.

Cold vs warm extraction.xlsx dataset.

This Excel workbook includes the full raw and analysed data for cold (C) and warm (H) feces extracts for each bile acid.

The data for each bile acid is provided in a separate sheet.

The variable names are given in square brackets.

- [Sample ID] is the unique identifier for each sample in the dataset.
- [Height] is the height of the detected chromatographic peak.
- [Area] is the area under the curve for the detected chromatographic peak.
- [ISTD Response] is the area under the curve for assigned internal standard.
- [Response Ratio] is the peak area ratio of each analyte to the internal standard.
- [Calculated Amt] is the calculated concentration for each analyte.

2. Experimental Design, Materials and Methods

2.1. Bile acids included in study

The following bile acids were measured: cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), glycodeoxycholic acid (GDCA), glycocholic acid (GCA), glycolithocholic acid (GLCA), taurochenodeoxycholic acid (TCDCA), taurocholic acid (TCA), taurodeoxycholic acid (TDCA), tauroursodeoxycholic acid (TUDCA).

The following deuterated bile acids were used as internal standards for the study: lithocholic acid (2,2,4,4-d4) (LCA-d4), taurodeoxycholic acid (2,2,4,4-d4) (TDCA-d4), deoxycholic acid (2,2,4,4-d4) (DCA-d4), and chenodeoxycholic acid (2,2,4,4-d4) (CDCA-d4).

2.2. LC-MS/MS method development

Optimization of MS parameters was performed on an ESI source. The arbitrary units including sheath, auxiliary, and sweep gasses were set at 35, 15, and 0, respectively. The ion transfer and vaporizer temperatures were set at 325 and 275 °C, respectively. The detection was performed in negative mode (~ 2500 V) and the spectra were acquired in multiple reaction monitoring (MRM) mode. The optimal LC separation was achieved on ACE C18 (100 mm × 2.1 mm ID; Advanced Chromatography Technologies, Scotland) with 1.7 mm particles and a mobile phase of water containing 0.1% formic acid (A) and acetonitrile (B). The gradient separation was completed in 14 min with the initial conditions of 99.9% solvent A and 0.1% solvent B [1].
2.3. Optimization of bile acids extraction from fecal sample

2.3.1. Optimized extraction of wet feces

Fecal samples were homogenized, and aliquots (approximately 0.5 g, accurately weighed) were transferred into 2 mL screw top storage tubes and stored at −80 °C until analysis. Fecal aliquots were thawed and extracted with 1.00 mL ice-cold methanol containing internal standards (5000 nM of TDCA-d4 and CDCA-d4, and 10,000 nM of LCA-d4 and DCA-d4). The sample was shaken for 30 min at 4 °C and centrifuged at 21,000 rpm for 20 min. The supernatant (100 μL) was transferred to a separate 1.5 mL microcentrifuge tube and diluted (1:5 v/v) with 0.1% aqueous formic acid solution. The extract was then filtered (0.22 μm polypropylene syringe) and transferred to a glass insert housed in a 2 mL amber glass LC vial ready for analysis.

2.3.2. Extraction of wet versus dry fecal samples

A pooled fecal sample was prepared from 6 individual wet fecal samples and from the pooled sample 12 (0.5 g each) aliquots were prepared. The aliquots were treated as follow: 4 aliquots (D) were dry-lyophilised using a freeze dryer; a further 4 aliquots (SD) were spiked with deuterated internal standards (50 μL of a solution containing LCA-d4 (10,000 nM), DCA-d4 (10,000 nM), CDCA-d4 (5000 nM), TDCA-d4 (5000 nM) and then dry-lyophilised, and another 4 aliquots (W) were frozen at −80 °C until further analysis. All fecal samples were extracted using optimized extraction procedure (see Section 2.3.1 above) and the level of bile acids in D, SD, and W samples were compared [1].

2.3.3. Stability

For the temperature stability study, 5 bile acid standard solutions (SS1 to SS5*) and 4 fecal extracts (labelled Ext 1, Ext 2, Ext3, Ext 4) were stored at: room temperature (22–25 °C) and at 6 °C in the autosampler for up to 24 h. All samples were analysed at baseline (t = 0), t = 12 h and t = 24 h, and percentage relative recovery calculated [1].

For the freeze-thaw stability study, 5 bile acid standard solutions (SS1 to SS5*) and 4 fecal extracts (Ext 1 to Ext 4) were exposed to three −80 °C freeze-thaw cycles. All samples were analysed at baseline (fresh samples) and after each of a total of 3 freeze-thaw cycles, and percentage relative recovery was calculated [1].

*SS1 to SS5 concentrations were 195.31, 781.25, 3125, 12,500, and 50,000 nM for CA, GCA, GDCA, GLCA, TCA, TCDDA, TDCA, TUDCA and UDCA, 781.25, 3125, 12,500, 50,000 and 200,000 nM for CDCA, and 2343.75, 9375, 37,500, 150,000, and 600,000 nM for LCA and DCA, respectively.

2.3.4. Cold vs warm extraction

Six fecal samples were extracted at two temperatures (4 °C and 37 °C) and the mean recovery of the bile acids from the cold extraction was compared to the warm extraction.

Ethics Statement

This study has been approved by the Edith Cowan University Human Research Ethics Committee (HREC) (ID 15362: Newton). All participants were provided with either a digital or hard copy written consent form which they signed prior to commencement in the study.

CRediT Author Statement

Armaghan Shafaei: Conceptualization, Methodology, Investigation, Validation, Writing original draft; Joanna Rees: Resources, Investigation, Writing original draft; Claus T. Christophersen:
Conceptualization, Resources, Writing review & editing; **Amanda Devine**: Conceptualization, Resources, Writing review & editing; **David Broadhurst**: Formal analysis, Visualization, Writing review & editing; **Mary C. Boyce**: Methodology, Conceptualization, Supervision, Writing review & editing.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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**Reference**

[1] A. Shafaei, J. Rees, C.T. Christophersen, A. Devine, D. Broadhurst, M.C. Boyce, Extraction and quantitative determination of bile acids in feces, Anal. Chim. Acta. (2021) 1150, doi:10.1016/j.aca.2021.338224.