

Abstract

The bilin tetrapyrrole stercobilin is a metabolite unique to mammalian waste.¹ Prior work has utilized stercobilin as a marker for both water contamination as well as a diagnostic for human health^[2-4]. Research in our group has found that the metabolite is depleted in the urine of autistic children versus controls. We now wish to determine if this depletion is also observable in animal models of autism. Since commercial isotopomers for bilins are currently unavailable, we have developed a method to produce stercobilin isotopomers to be utilized for the quantification of Stercobilin by mass spectrometry.⁵

Introduction

What is autism?

- Autism spectrum disorder (ASD) and autism are both general terms for a group of complex disorders of brain development.⁶
- ASD can be associated with intellectual disability, difficulties in motor coordination and attention, and physical health issues such as sleep and gastrointestinal disturbances⁶

What is Stercobilin?

- Stercobilin (C₃₃H₄₆N₄O₆) is a member of the group of mammalian metabolites known as bilin tetrapyrroles.¹
- Stercobilin is a product of heme metabolism formed from the degradation of bilirubin.¹

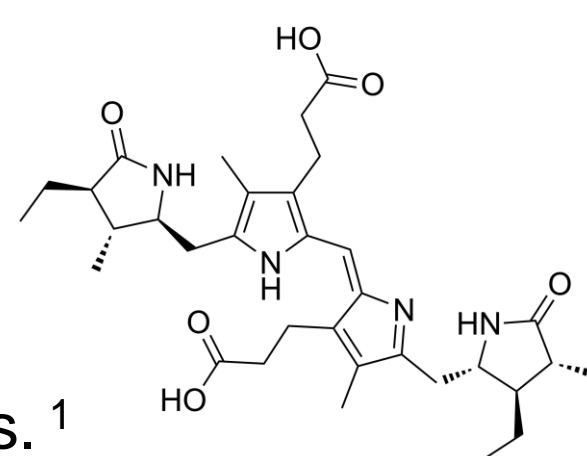


Fig.1: Structure of Stercobilin

Methods

Instrumentation

- All analysis is done by a 12T Bruker Solarix FT-ICR MS



Fig. 2: 12T Bruker Solarix FT-ICR MS

Reaction Time, Temperature and Volume Dependence

- Reproducibility evaluated by highest yield with acceptable relative standard deviation.
- Each trial was performed three times to compare the reproducibility
- Temperatures:** 50° C, 60° C, and 70° C.
- Times:** 1, 4, and 8 hours.
- Volume of H₂¹⁸O:** 5 µL or 10 µL

Urinalysis

- Urine samples from volunteers were analyzed by SPE and MS to find the concentration of Stercobilin.

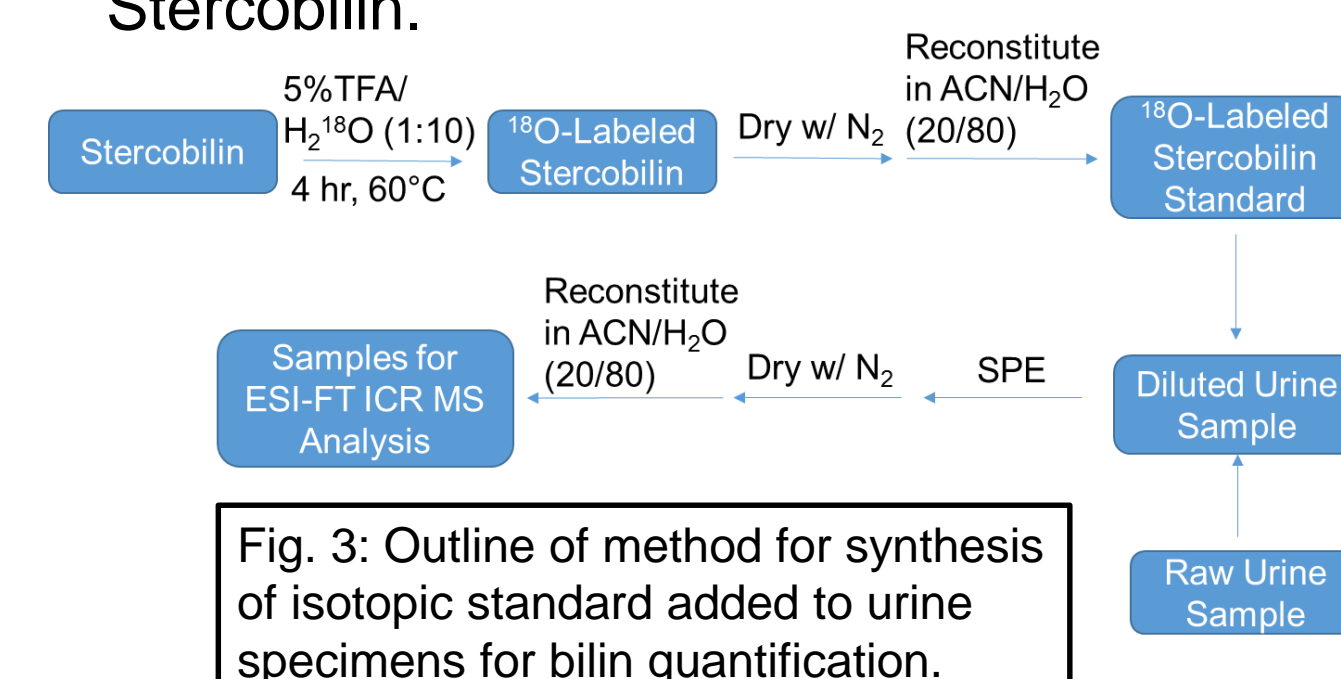


Fig. 3: Outline of method for synthesis of isotopic standard added to urine specimens for bilin quantification.

Timothy Syndrome Mice

- Timothy Syndrome in mice has shown to be an accurate animal model for autism.⁷
- Supernatant from fecal collections are utilized to obtain statistical data of the concentration of stercobilin within each sample.

Results

Reaction Time, Temperature and Volume Dependence

- Best reproducibility occurred at 60° C when the sample was left for 4 hours with 10 µL of H₂¹⁸O

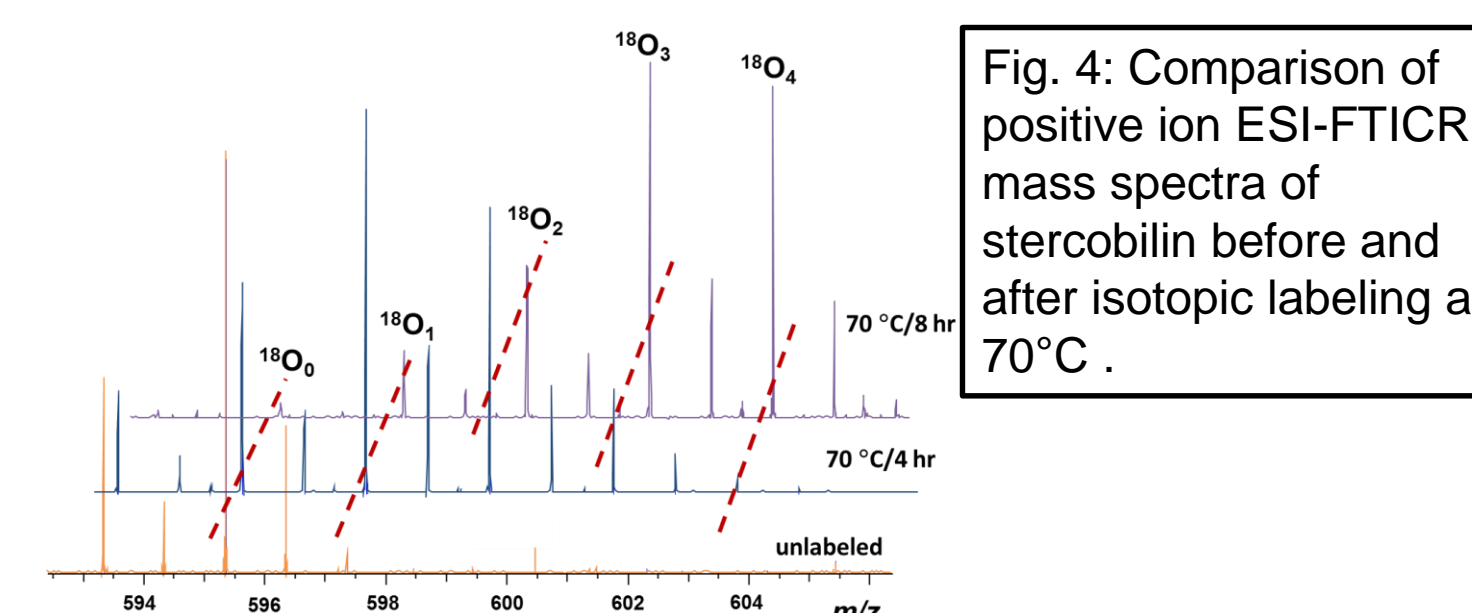


Fig. 4: Comparison of positive ion ESI-FTICR mass spectra of stercobilin before and after isotopic labeling at 70°C.

Urinalysis

- Preliminary results show that on average females excrete lower levels of stercobilin than males.
- Further examination of a larger sample population will need to be completed.

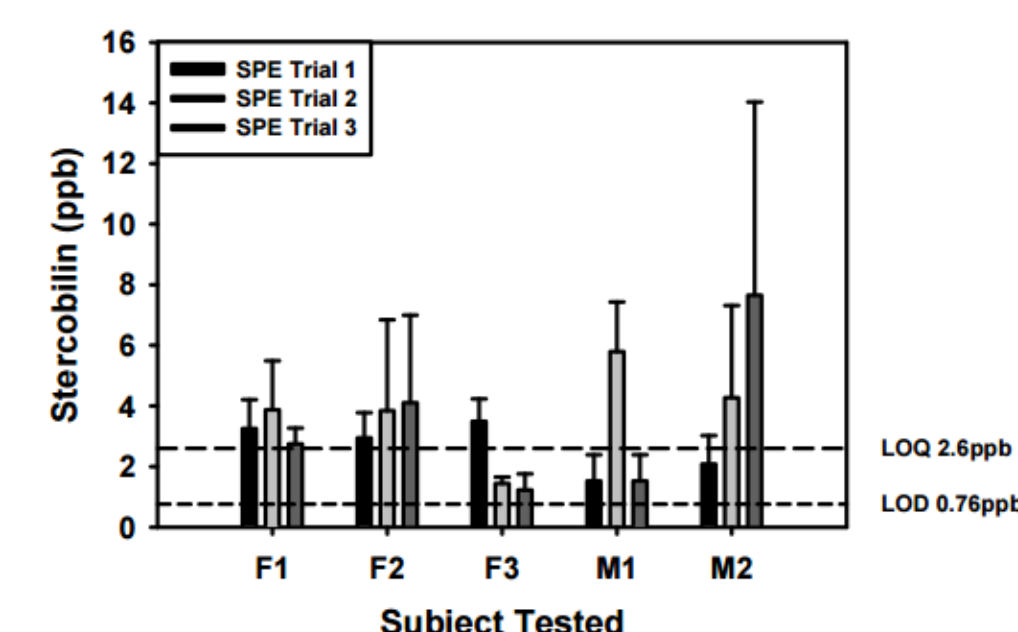


Fig. 5: Stercobilin concentration comparison via urinalysis among tested subjects.

Timothy Syndrome Mice

- Preliminary results show that control mice exhibit a higher concentration of stercobilin per mass of feces compared to Timothy Syndrome mice.
- More samples need to be collected and tested for each set of mice.

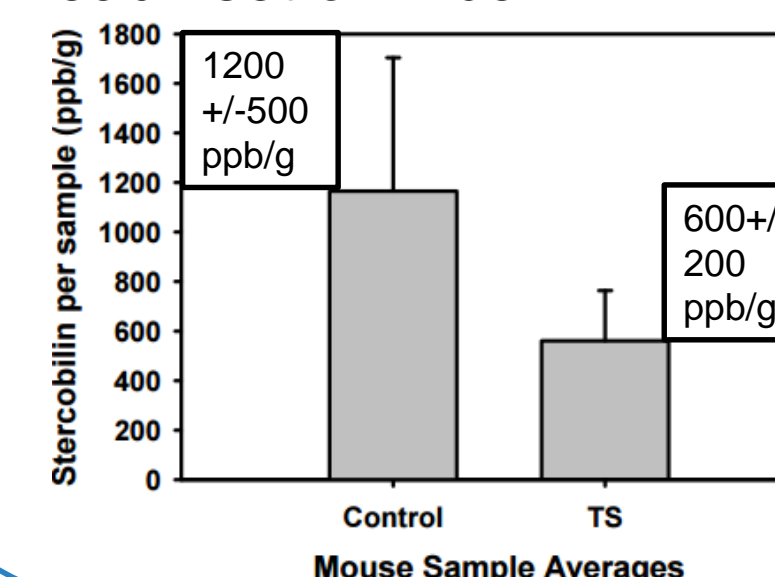


Fig. 6: Stercobilin concentration comparison between Timothy Syndrome mice and control matched littermates.

Future Work

Double Incubation of ¹⁸O Stercobilin

- By completing incubation twice, we may see higher labelling of stercobilin by four ¹⁸O's.

Completion of Animal Model Work

- More samples from both control and Timothy syndrome mice should be completed to prove our model.

Development of ASD Screening Method

- With new samples from autistic and control subjects we can begin to:
 - Prove if our hypothesis holds true in humans
 - Develop a screening method for ASD

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