Protocol for polar metabolomics (with emphasis on thyreostats) of urinary samples from cattle

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Abstract

This protocol describes the untargeted polar analysis of urine samples from cows and calves, with special emphasis on the coverage of thyreostats. Extraction started with 3 mL urine and was in essence based on liquid-liquid extraction with ethyl acetate. Analysis of extracts was achieved by ultra-high performance liquid chromatography (UHPLC) using a Waters Acquity HSS T3 column (1.8 µm, 2.1 x 100 mm), HESI-II ionization, and Q-Exactive Orbitrap mass spectrometry.

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Guidelines

Collected urine samples were treated with EDTA (final concentration of 0.1 M) and 0.1 M hydrogen chloride (final pH of 1) to inhibit thyreostat degradation during storage (-20 °C).

The phosphate buffer was adjusted to a pH of 7.

Before start

Frozen urine samples were thawed at 4 °C before extraction could be started.

Protocol

Urine starting volume

Step 1.

AMOUNT

3 ml Additional info: Urine

Pretreatment

Step 2.

AMOUNT

50 ng Additional info: Propylthiouracil-d5 internal standard

AMOUNT

1 ml Additional info: Phosphate buffer (containing 1% DL-dithiothreitol)

Denaturation

Step 3.

TEMPERATURE

65 °C Additional info:

Liquid-liquid extraction

Step 4.

AMOUNT

5 ml Additional info: ethyl acetate

AMOUNT

5 ml Additional info: ethyl acetate

NOTES

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Collect the supernatans in a separate recipient

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Collect the supernatans in the previously mentioned recipient.

Concentrating the extract

Step 5.

AMOUNT

200 µl Additional info: Dissolve the residue in ultra pure water (0.1% formic acid)/methanol (0.1% formic acid) (90/10, v/v)

TEMPERATURE

60 °C Additional info:

NOTES

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Evaparation under a gentle stream of nitrogen until dry