UHPLC-MS/MS ANALYSIS OF THYREOSTATS IN BOVINE FAECES

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INTRODUCTION

Thyreostats are tioamid antithyroid drugs. Activity of these compounds consists in inhibiting the synthesis of thyroid hormones triiodothyronine (T3) and thyroxine (T4), which favors the processes of animal fattening. Increase in weight of animals is mainly due to the water retention in the tissues and the gastrointestinal tract. According to the International Agency for Research on Cancer some compounds of this group possess carcinogenic and teratogenic properties. For this reason the use of thyreostatic drugs for animal fattening purposes is banned in the European Union since 1981. In accordance with the Council Directive 96/23/EC thyreostats belong to the group A2 - compounds with anabolic properties, which must be controlled in slaughter animals.

The aim of the study was to develop fast and simple method for the detection of thyresostats in faeces matrix.

MATERIAL AND METHOD

SAMPLES

The faeces samples free of thyreostats were fortified with mix of TAP, TU, MTU, DMTU, PTU, PhTU at concentrations of 5, 10, 15 µg kg^{-1}. To each sample 10 ng of internal standards were added (TAP-d3, PTU-d5) to a concentration of 20 µg kg^{-1}.

SAMPLE PREPARATION

Faeces (0.5 g) + 10 mL methanol/ Britton-Robinson buffer (2:8, v/v)

Mixing (1 min) Centrifugation

5ml of organic phase transfer to new tube

Derivatisation 100 µL IBBR (40 °C, 60 min)

Adjusting pH to 4.5

LLE 2 x 3mL diethyl ether

Evaporation, 40°C

Mobile phase (500µL) ACN / 0.1% acetic acid (20:80, v/v)

UHPLC-MS/MS

	SHIMADZU NEXERA X2	8050 Mass Spectrometer
Ionization	Source type: ESI Polarity: Positive Scan Type: MRM	Interface Temperature: 300°C
Column	Agilent ZORBAX SB-C18 (50 mm x 2.1 mm, 1.8 µm), 45°C	
Mobile phase	Acetonitrile / 0.1% acetic acid aqueous solution (20:80, v/v) Flow rate: 0.6 mL min^{-1}	
Injection volume	15 µL	
Run Time	7.5 min	

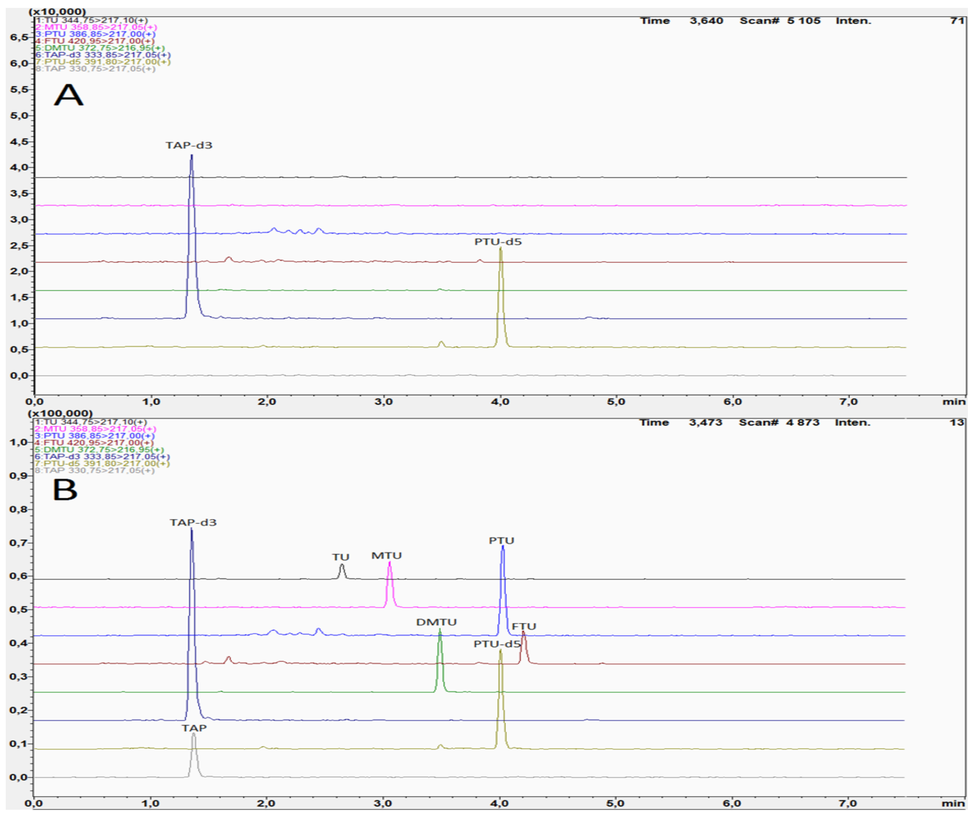
RESULTS

VALIDATION

The validation of the method was performed according with Commission Decision 2002/657/EC requirements.

Overview of validation results at 10 µg L^{-1} concentration level

Analyte	Recovery (%)	Repeatability (CV, %)	Reproducibility (CV, %)	CCα (µg kg^{-1})	CCβ (µg kg^{-1})
TAP	97.5	5.9	9.5	3.26	6.52
TU	112.7	4.4	7.1	2.21	4.42
MTU	113.5	10.3	16.5	2.99	5.99
PTU	101.6	11.1	17.8	3.29	6.58
PhTU	112	14.1	22.6	3.48	6.96
DMTU	98.7	13.6	21.8	3.00	6.01



LC-MS/MS chromatograms of faeces samples:
A – blank sample; B – sample spiked at 5 µg kg^{-1}.

CONCLUSION

This work presents a simple and easy LC-MS/MS method that simultaneously identifies and quantifies the following six thyreostats in faeces samples: tapazole, thiouracil, methylthiouracil, dimethylthiouracil, propylthiouracil and phenylthiouracil. The method consists an easy sample preparation procedure based on the derivatisation and only the liquid-liquid extraction. The use of a UPLC ZORBAX C-18 column allowed for a very good chromatographic separation of analytes and short LC- MS/MS running time. The method is sensitive to all estimated CCα and CCβ values lower than the recommended concentration for urine set at 10 µg L^{-1}.

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