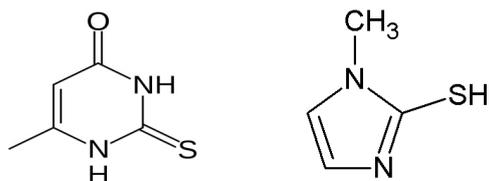


Proficiency test Thyrostats in samples of porcine urine

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Introduction

Thyrostatic compounds are orally active drugs that may be used to increase the weight of pigs before slaughter. This weight gain is mainly due to increased water absorption and retention within edible tissues as well as filling of the intestinal tract by inhibiting thyroid hormone production. The use of this group of compounds leads to the production of meat of lower quality and is considered as an abuse, because water is sold for the price of meat. In particular, 6-methyl-2-thiouracil (MTU) and 1-methyl-2-mercapto-imidazole (TAP) are very strong thyroid-inhibiting compounds which are inexpensive and easy to obtain. This study was organised in December 2007 by the EU-RL. The purpose of this study was to evaluate current analytical methodology for the quantification of TAP and MTU in samples of porcine urine, as performed by EU NRLs.



The molecular structure of MTU and TAP.

Animal Experiment

An important requirement for proficiency tests is the use of incurred material in order to create conditions which are as similar as possible to real-life samples. For this proficiency test, we used samples of urine obtained from three pigs (± 12 months old). The animals were treated by oral administration with TAP and MTU on day 0 and day 4 (approximately 40 mg per treatment). MTU and TAP were excreted into samples of urine during the days following treatment of the animals. Samples of urine were collected on days 0, 1, 2, 3, 4, 5, 6, 7 and stored in a freezer at -20°C until analysis.

Production of the samples

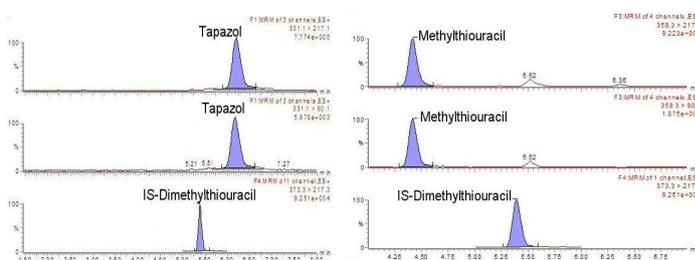
Selected samples were blended with blank porcine urine to obtain target concentrations. Each sample was numbered with a unique number generated with the management information system.



Analytical methods

Most laboratories prefer the use of LC-MS/MS. At the EU-RL, the clean-up consist: hydrolysis of the samples, derivatisation with 3-iodobenzylbromide at pH 8, liquid-liquid extraction (LLE) with ethyl-acetate and liquid chromatography (LC) in combination with mass spectrometric detection (LC-MS/MS).

The chromatograms below show the masses used for the detection of TAP and MTU in a sample of porcine urine. The internal standard was Dimethylthiouracil (DMTU).



Conclusion and recommendations

In total 26 laboratories registered as participant for the test and 25 laboratories reported their results. A majority of participants (19 labs) was able to quantify and confirm MTU and TAP in samples of porcine urine. The evaluation of the quantitative results is based on Z-scores.

Summary of the total number of results (MTU)

Sample code	Number of participants	Analysed	No results	Outlier	$0 \leq Z \leq 1$	$1 \leq Z \leq 2$	$ Z > 2$
Batch A	26	25	1	1	19	2	3
Batch B	26	25	3	0	18	2	2
		Min (ppb)	Max (ppb)	Mean (ppb)	Median (ppb)	s.d.	CV%
Batch A		46.0	212.0	86.33	75.00	26.17	30
Batch B		8.00	46.3	21.34	20.00	8.54	40

Summary of the total number of results (TAP)

Sample code	Number of participants	Analysed	No results	Outlier	$0 \leq Z \leq 1$	$1 \leq Z \leq 2$	$ Z > 2$
Batch A	26	22	4	1	4	11	6
Batch B	26	20	6	2	9	6	3
		Min (ppb)	Max (ppb)	Mean (ppb)	Median (ppb)	s.d.	CV%
Batch A		11.10	301.0	47.31	31.50	59.30	125
Batch B		3.02	72.53	15.75	11.36	13.36	85

- Batch A. - This sample was focused on 50 ppb MTU and TAP. The overall average value of obtained during the test was, for MTU 86 ppb (S.D. 26) and for TAP 45 ppb (S.D. 61), with no observed difference between day one and day 2.
- Batch B. - This sample was focused on 10 ppb MTU and TAP. The overall average value of obtained during the test was, for MTU 22 ppb (S.D. 8) and for TAP 15 ppb (S.D. 14), with no observed difference between day one and day 2.

On average the performance is acceptable. However, especially for TAP, some laboratories should improve their performance.