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# DETERMINATION OF NITROIMIDAZOLES AND THEIR METABOLITES IN MILK USING GC/MS-NCI

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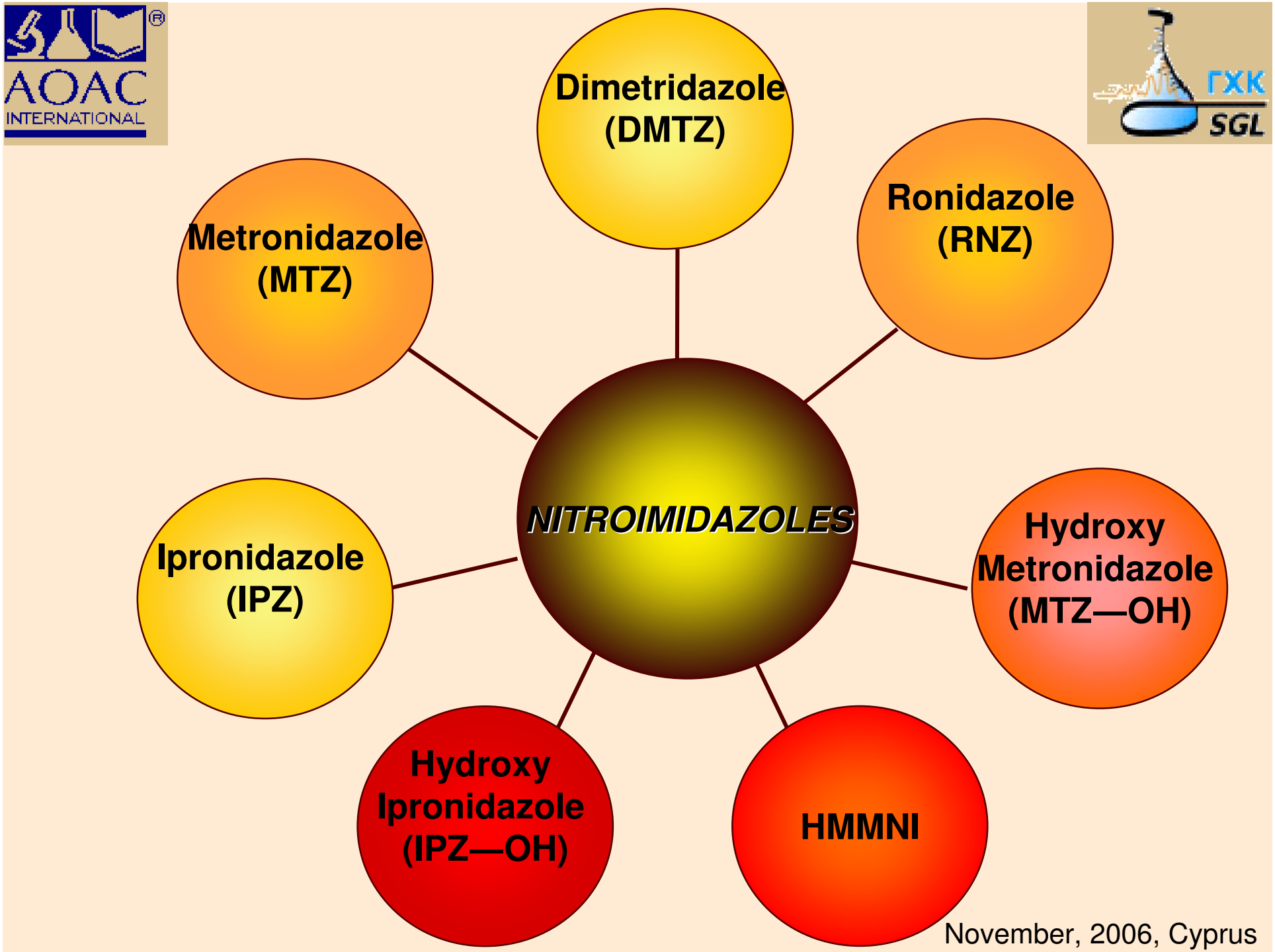
# Introduction

According to the directive 96/23/EC, the use of Nitroimidazoles in animals is prohibited. Consequently, analytical strategies are needed for monitoring their use through analysis of samples of animal origin.

Milk, as a product of animal origin, is of prime concern due its consumption by a vulnerable part of the population, namely children. A method was therefore developed and validated for the determination of Nitroimidazoles in milk. An internal standard for each nitroimidazole was used.


Dimetridazole
(DMTZ)
Metronidazole
(MTZ)
Ronidazole
(RNZ)
NITROIMIDAZOLES
Ipronidazole
(IPZ)
Hydroxy
Metronidazole
(MTZ—OH)
Hydroxy
Ipronidazole
(IPZ—OH)
HMMNI

# Introduction

The estimated value of $CC_\alpha$ has been found to be between 0.14–0.74 μg/l which is lower than the proposed MRPL (Minimum Requirement Performance Limit) (3 μg/l), so the method has been proved to be appropriate to use for the analysis of Nitroimidazoles.

The method provides a tool to Competent Food Authorities/National Reference Laboratories to monitor the abuse of this class of veterinary drugs and thus attaining one more step towards the further improvement of public health protection.

Milk (4 ml)

Internal standard added

Centrifugation

Fat layer discarded

Buffer solution added (pH 3) to vol. of 8 ml,
1 ml of protease added

Thorough mixing/adjusted to pH 2
using 2M HCl

Hydrolyze phase for at least 2 hr or overnight
at 37°C

pH adjusted to 3

Centrifugation

Hexane for defatting (10 ml),
centrifugation

Hexane phases discarded

The above step is repeated (using 5 ml)

pH 6 adjusted with 5M NaOH, filled up to 8 ml

XTR cartridges used (20 min waiting time), 9 ml of ethyl acetate/tBME
(1:1) added, elution with 2×9 ml of ethyl acetate/tBME (1:1)

Concentration to dryness, glass vial rinsed twice with 0.5 ml of ethyl
acetate/tBME (1:1)

Combined extracts evaporated to dryness

BSA/n-heptane 1:1 (v:v) added

Derivatisation for 1 hr at 50°C, 1 µl injection
into GC-MS

# Instrumentation

- Agilent GC 6890N
- MS-NCI detector 5973-inert[1]

Parameters of the GC-MS/NCI:

- Analytical Column HP-5MS (30 m×0.25 mm×0.25 μm)
- Precolumn: Fused Silica Intermediate Polarity (1 m×0.32 mm)
- Reagent Gas: Methane
- Carrier Gas: Helium
- In. Temp.: 85° C
- In. Time: 1.50 min

# Validation

The method was validated according to the European Union Decision 657/2002/EC.[2]

[1] Confirmatory Method for the Determination of Nitroimidazoles in Plasma using GC—MS/NCI. Polzer, J., Gowink, P., Code: NIIM-007, CRL/BVL Berlin, 1/3/2002.

[2] Official Journal of the European Communities L221, 8—36, Commission Decision (2002/657/EC) of 12 August 2002 Brussels, Belgium, 20.

# Results and Discussion

Due to matrix interferences the standard curve of spiked samples was used for quantification. Four levels of spiking were used:1, 2, 3, 4 μg/l in order to evaluate:

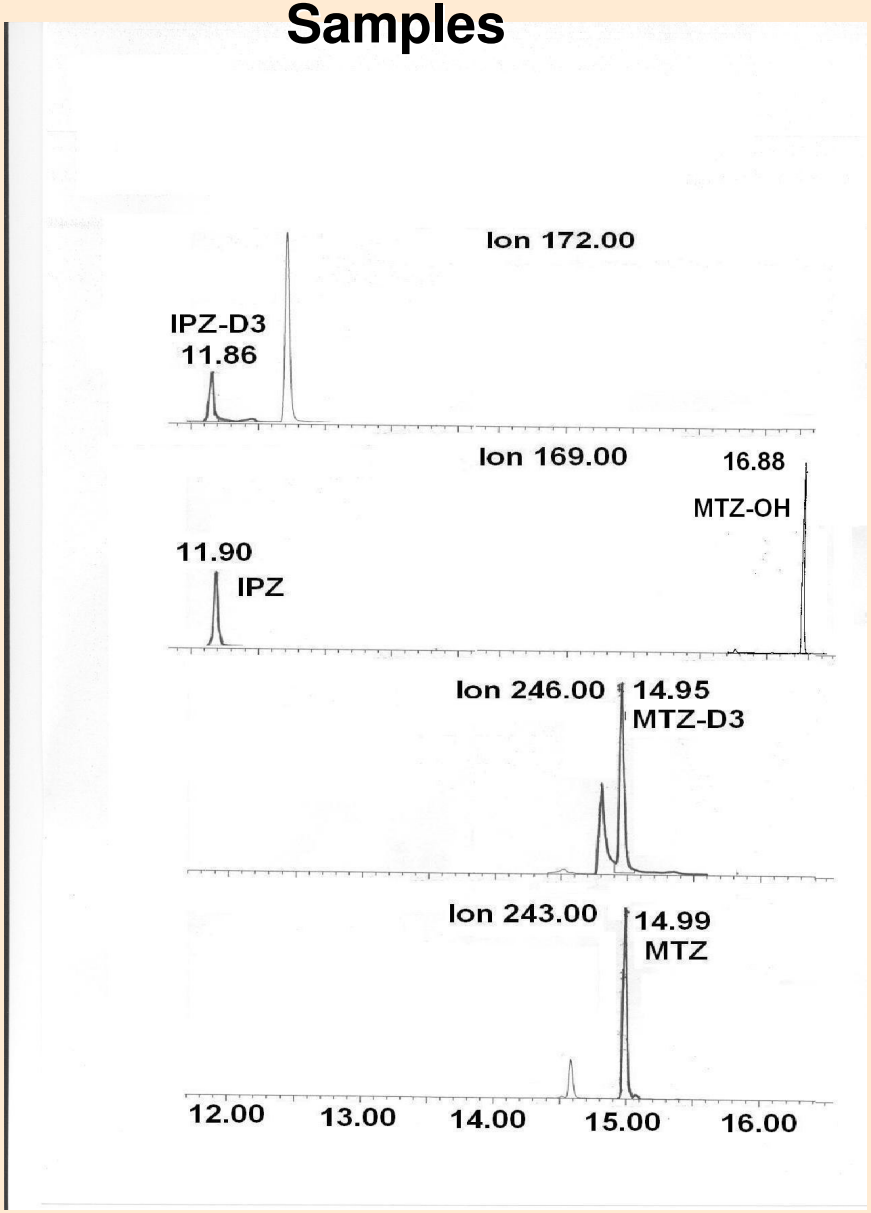
- Decision limit ($CC_\alpha$)
- Detection capability ($CC_\beta$)
- Repeatability
- Reproducibility
- Recovery

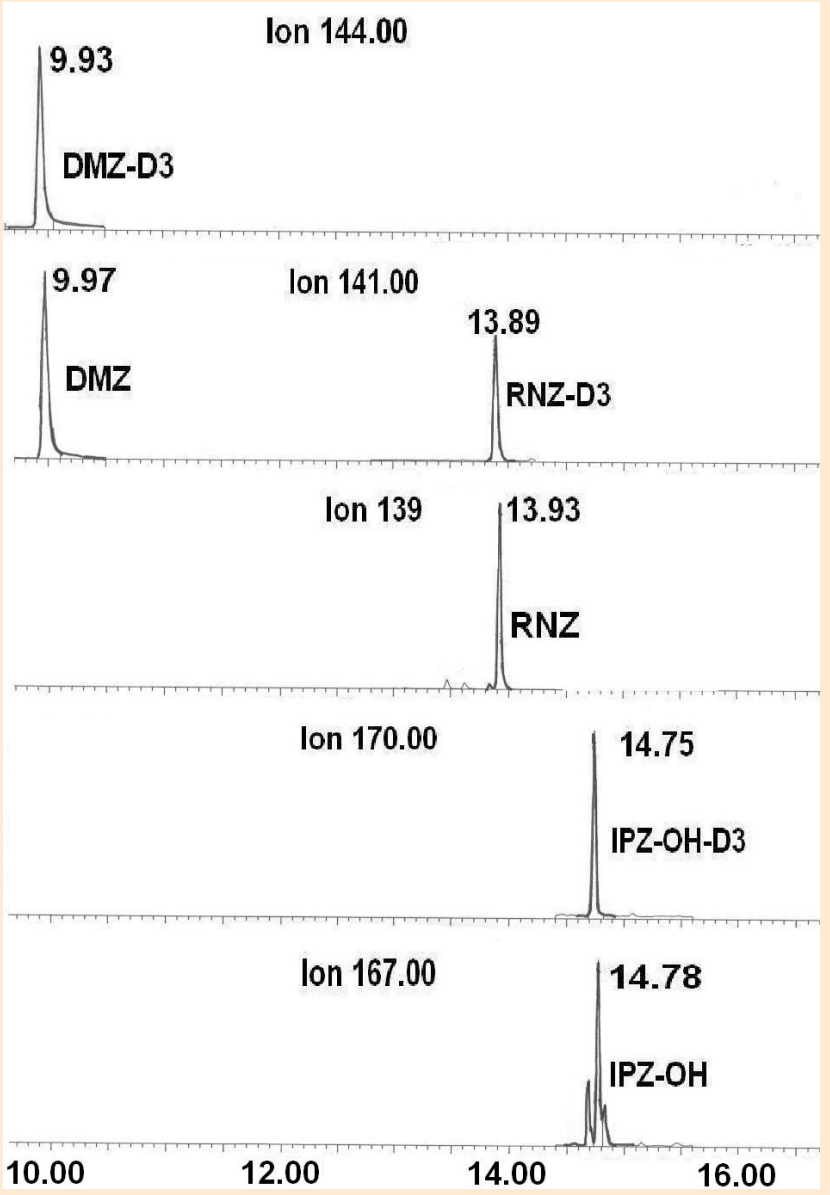
**ISO/IEC 17025**

# Validation Data

| | $CC_\alpha$ (µg/l) | $CC_\beta$ (µg/l) | Rep/ty (%RSD, n=24) At MRPL | Reprod/ty (%RSD, n=24) at MRPL | Recovery (%) |
|---|---|---|---|---|---|
| RNZ | 0.56 | 0.85 | 4.6 | 9.9 | 101 |
| MTZ | 0.16 | 0.36 | 6.0 | 7.5 | 102 |
| DMZ | 0.29 | 0.49 | 7.3 | 14.4 | 107 |
| IPZ | 0.14 | 0.34 | 10.5 | 15.9 | 100 |
| MTZ-OH | 0.74 | 1.29 | 15.8 | 28.5 | 77 |
| IPZ-OH | 0.54 | 0.81 | 7.3 | 13.9 | 111 |

# Chromatographic Results from Spiked Samples

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# Conclusions

Quantities of nitroimidazoles are not affected by discarding the fat layer.

The method is a multiresidue method and can be applied in routine laboratory processes.

The determined *CCα*, *CCβ* are more than satisfactory.

Precision and recoveries were well resolved.

# *Thanks for Listening*

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