DETERMINATION OF NITROIMIDAZOLES USING GC/MS, GC/NCI AND GC/MS/MS

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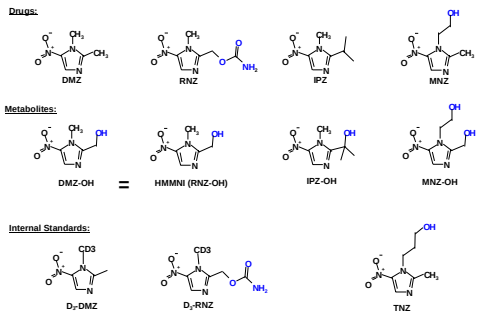
Introduction

Since the ban of ronidazole (RNZ) in 1993 two further nitroimidazoles have been banned for the use as veterinary drugs by the European Commission and must be monitored in compliance with the residue control plans. RNZ, dimetridazole (DMZ) and metronidazole (MNZ) being banned substances and ipronidazole (IPZ) being a non authorized one, their monitoring requires state-of-the-art methods especially concerning analytical sensitivity and effectivity.
The presented method allows the analysis of samples of turkey and pig muscle for residues of the most frequently used nitroimidazoles as well as their main hydroxy metabolites having retained the origina[1] nitroimidazole ring structure.

Analytes and Internal Standards



Sample Preparation

1-10 g of a minced tissue sample
- ⇨ addition of buffer (pH 3) and protease solution (type XVIII, Sigma), homogenisation by means of a stomacher
- ⇨ hydrolysis overnight
- ⇨ centrifugation and separation of the aqueous layer
- ⇨ defattening with hexane.
- ⇨ pH-adjustment to 6 and application to an extrelut cartridge (NT20, Merck, Germany)
- ⇨ extraction with tert.-butylmethylether and ethyl-acetate (1:1)
- ⇨ evaporation to dryness and derivatisation with N,O-bis-(trimethylsilyl)acetamide (BSA)/$CHCl_3$) (RNZ and HMMNI produce the same derivative!)
- ⇨ Injection of 1-2 µl of this mixture into the GC.

Equipment

Finnigan GCQ (ion trap), NCI and MS/MS
30 m x 0,25 mm ID, 0,25 µm DB5 column
(for analysis)
Hewlett Packard GC/MSD 6890/5973, EI/MS,
30 m x 0,25 mm ID, 0,25 µm DB1 column

GC/MS-Analysis

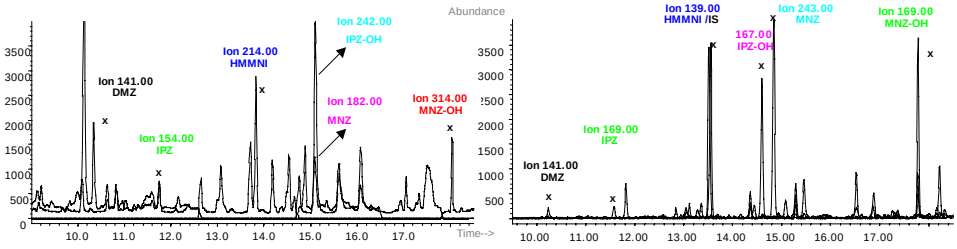
Injector : 285° C, 1.5 min splitless time
Temp. progr.: 60°C for 1.5 min,
12° C/min to 144°C,
5° C/min to 184° C,
25° C/min to 290° C, 6 min.

EI : SIM (ions see table 1)
NCI : methane at $1.1*10^{-4}$ torr, full scan
MS/MS : Dual mode, base ions as precursor

Results

EI versus NCI detection:

representative ion chromatograms of a spiked sample of turkey muscle (relevant peaks cross-marked, 10 ppb of each analyte in 10 g muscle):



EI/MS (DB1 column) NCI/MS (DB5 column)
a DB5 column allows for a chromatographic separation of IPZOH and MNZ!

Recoveries of the nitroimidazoles with EI/MSD detection (values for 10 ppb, 10 g of matrix)

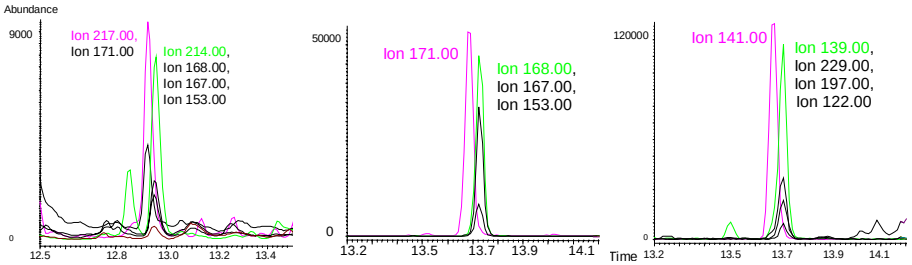
Analyte	Ions (SIM)	Recovery	SD*	LOD**
DMZ	(141, 112, 98)	102,5 %	+/- 9,6	≈ 5 ppb
IPZ	(169, 154, 123)	75,4 %	+/- 12,8	≈ 5 ppb
RNZ	(214, 168, 167, 153)	91,5 %	+/- 10,2	≈ 2 ppb
IPZOH	(242, 226, 196, 184)	113,6 %	+/- 25,6	≈ 5 ppb
MNZ	(228, 197, 182, 167)	104,1 %	+/- 21,0	≈ 5 ppb
MNZOH	(316, 315, 314, 272)	83,9 %	+/- 9,0	≈ 2 ppb

* standard deviation, n=10
** limit of detection, estimated for a s/n ratio of 5 for the base ion (depends on matrix effects for EI/MS)

Comparison of quantitative results for RNZ in spiked matrix samples (D_3-RNZ / RNZ at 10 ppb each) using EI/MS, NCI/MS and MS/MS

D_3-RNZ / RNZ ions (IS / analyte)	EI/MS (171/214)	NCI/MS (141/139)	EI/MSMS (171/169)
standard calibr.			
recovery (n=4)	84 % +/- 9	93 % +/- 4	91 % +/- 5
slope y =	4,76x-0.144	1.669x-0.123	1.168x-0.003
correlation r =	0.997	0.994	0.995
matrix calibr.			
recovery (n=4)	107 % +/- 5	97 % +/- 4	102 % +/- 6
slope y =	3,24x-0.063	1,719x-0.093	1,030x-0.003
correlation r =	0.998	0.995	0.997

Comparison of ion traces of D_3RNZ / RNZ (spiked matrix sample at 7.5 ppb) in EI/MS, EI/MS/MS and NCI/MS detection mode



EI/MS EI/MS/MS NCI/MS

Summary

The analytical method allows for
- sensitive screening of DMZ, IPZ, MNZ, IPZ and their main metabolites in matrix samples;
- confirmatory analysis (e.g by combining different MS-techniques);
- particularly satisfactory analytical results using NCI or MS/MS techniques due to
  - less interferences from matrix effects
  - lower detection limits;
- high accuracy and precision with respect to the determination of DMZ and RNZ and their common metabolite HMMNI (since adequate internal standards are available).

Further analytical progress with respect to precision and accuracy for the derermination of the other nitroimidazoles will depend on the availability of the corresponding isotope-labelled substances.

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References:
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4. S.Semeniuk, A.Posyniak, J.Niedzielska, J. Zmudzki, *Biomed. Chromatogr.* **9** (1995), 238.