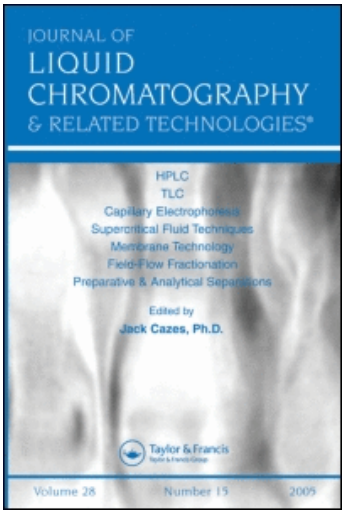

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF NITROIMIDAZOLE RESIDUES IN MEAT USING MASS SPECTROMETRY

Victor Hormazábal[a]; Magne Yndestad[a]
[a] Division of Food Hygiene, The Norwegian School of Veterinary Science, Oslo, Norway

Online publication date: 30 September 2001

To cite this Article Hormazábal, Victor and Yndestad, Magne(2001) 'DETERMINATION OF NITROIMIDAZOLE RESIDUES IN MEAT USING MASS SPECTROMETRY', Journal of Liquid Chromatography & Related Technologies, 24: 16, 2487 — 2492
To link to this Article: DOI: 10.1081/JLC-100105954
URL: http://dx.doi.org/10.1081/JLC-100105954

J. LIQ. CHROM. & REL. TECHNOL., 24(16), 2487–2492 (2001)

DETERMINATION OF NITROIMIDAZOLE RESIDUES IN MEAT USING MASS SPECTROMETRY

Victor Hormazábal and Magne Yndestad*

Division of Food Hygiene, The Norwegian School of Veterinary Science, P. O. Box 8146-Dep., N-0033 Oslo, Norway

ABSTRACT

A method was developed for the determination of the nitroimidazole compounds dimetridazole, metronidazole, and ronidazole in meat. Meat was extracted with acetonitrile and the organic fraction was separated from the aqueous with sodium chloride and dichloromethane. Water was added, the organic layer was evaporated, and fat was extracted with hexane. The water residue was injected into the LC/MS. The lower limit of quantification was 1, 2, and 4 ng/g for dimetridazole, metronidazole, and ronidazole, respectively.

INTRODUCTION

Dimetridazole (DMZ), metronidazole (MNZ), and ronidazole (RNZ) are nitroimidazole compounds, which are used in veterinary medicine for the treatment of genital trichomoniasis in cattle and haemorrhagic enteritis in pigs. In

*Corresponding author.

addition to their antimicrobial effects, nitroimidazoles are also useful growth promotors. These compounds, along with their metabolites that retain the nitroimidazole ring structure, are suspected carcinogens, which have been banned for use as veterinary drugs by the European Commission.(1)

RNZ was incorporated in the "List of Pharmacologically-Active substances for Which no Maximum Residue Limit Can be Fixed" (Annex IV) in January 1994, and DMZ in July 1996.(2) DMZ is, nevertheless, still licensed for prophylactic use in gamebirds and turkeys according to the Feed Additives Directive 70/524/EEC.(3) The possibility that accidental feed contamination or improper use may give rise to unwanted residues in broiler chickens or pigs is the background for the requirement for methodology to detect these compounds at trace levels in different food-producing animals.

Several methods have been published describing the determination of DMZ, MNZ, and RNZ.(4-7) These methods are, however, time-consuming or require the use of large quantities of chemicals reagents.

The purpose of the present study was to develop a rapid, simple, and specific method for the quantification and confirmation of DMZ, MNZ, and RNZ in meat by LC-MS.

EXPERIMENTAL

Materials and Reagents

Samples of meat from pig and chicken were used. All chemicals and solvents were of analytical or HPLC grade. DMZ, MNZ, and RNZ were supplied by Sigma Co. (St. Louis, MO, USA). Stock solutions (1.0 mg/mL) of DMZ, MNZ, and RNZ, and working standards (0.1 and 1 μg/mL) were prepared by dilution with methanol and stored in a refrigerator at +4°C. Spin-X micro-centrifuge tube filters (0.22 μm nylon) supplied by Costar (USA) were used for filtration.

Chromatographic Conditions

The analyses were performed with a Perkin-Elmer LC-MS system, consisting of a Series 200 quaternary pump and a Series 200 autosampler. The acquired data were entered into a Model 8500 Apple Power Macintosh and processed with either Multiview 1.4 or MacQuan 1.6 software packages (Perkin-Elmer) for spectral information and quantification data processing.

An API 100 LC-MS system (PE SCIEX) single quadrupole mass spectrometer with a Turbo-Ion Spray Inlet for the API LC-MS System was employed

for this study. The turbo probe of the instrument was maintained at 150°C and the flow-rate of air for the probe was 6 L/min. The LC-MS was set to collect multiple single-ion data in positive ion mode for the ions at *m/z* 142, 172, and 201 for DMZ, MNZ, and RNZ, respectively. The entrance electrode voltages were adjusted to provide the optimum overall intensities for the three molecular ions.

The optimum sensitivities for DMZ, MNZ, and RNZ were obtained with N_2 nebuliser gas flow set to 5 L/min, the curtain gas was 10 L/min, and the ion source was 4500V. For DMZ, the orifice was 20V and for MNZ and RNZ 10V. The ring for DMZ and MNZ was 300V and for RNZ was 330V, while the Quadrupole 0 was -10V for DMZ, MNZ, and RNZ.

A Waters Xterra RP18 column (stainless steel, 250 x 4.6 mm I.D. packed with 5 µm particles) was employed for determining DMZ, MNZ, and RNZ. The guard column was connected to an A -318 precolumn filter on line with an A -102X frits (Upchurch Scientific, USA). The mobile phase was 0.05% (0.5 mL/L) acetic acid in water-methanol (70:30). The pump was operated isocratically at a flow rate of 0.9 mL/min.

The LC eluent was split, post-column, beforehand, approximately 1:20 with a flow rate of 1 mL/min with water-methanol (1:1), so that ca. 50 µL flowed into the Ion-Spray ion source.

Sample Pretreatment

Volumes of 0.5 mL methanol or standard (the total volume should always be 0.5 mL) and 8.5 mL acetonitrile were added to 3 g of sample. The mixture was homogenized for approximately 6 sec. with an Ultra-Turrax TP 18/10 (Janke & Kunkel KG,Germany). After centrifugation for 5 min at 5000 rpm, 6 mL of supernatant (corresponding to 1.5 g sample) was transferred to a glass-stoppered centrifuge tube, followed by 1 g NaCl and 1 mL CH_2Cl_2.

The mixture was shaken vigorously for 20 sec and centrifuged for 3 min at 3500 rpm. The upper layer was transferred to a graduate glass-stoppered tube and after addition of 0.4 mL water, mixed and evaporated to 0.4 mL under a stream of air, using a Reacti-Term heating module at 37°C and a Reacti Vap evaporating unit (Pierce, Rockford, IL, USA). If necessary, the volume was adjusted to 0.4 mL with water.

A volume of 1 mL hexane was added to the water layer followed by vortex-mixing for 5 sec. After centrifugation for 2 min, the hexane layer then was discarded. This hexane washing was repeated. The aqueous phase was centrifuged for 2 min at 10000 rpm through a Spin-X centrifuge filter. Aliquots of the filtrate (100 µL) were injected into the LC-MS at intervals of 10 min.

Calibration Curves and Recovery Studies

The calibration curves for DMZ, MNZ, and RNZ were established by spiking muscle from pig with standard solutions to produce concentrations of 0.5, 1, 2, 5, 10, 20, and 30 ng/g. Duplicate samples were used.

The recovery rates were determined by comparing recoveries from spiked muscle and standard solutions.

The linearity of the standard curves for DMZ and MNZ was calculated using peak area measurements, and for RNZ using peak height measurements.

RESULTS AND DISCUSSION

The standard curves were linear in the investigated areas; from 0.5 to 30 ng/g for DMZ, from 1 to 30 ng/g for MNZ, and from 5 to 30 ng/g for RNZ in meat. The correlation coefficient for DMZ in muscle was 0.998, and for MNZ and RNZ 0.997 when using the external standard method. Table 1 shows the recovery and repeatabilities for DMZ, MNZ, and RNZ from muscle.

The recoveries of DMZ, MNZ, and RNZ from muscle were 83-85%, 84-85%, and 83-87%, respectively, with standard deviations of 1.6-2.4% for DMZ, 2.2% for MNZ, and 1.2-1.8% for RNZ.

Our chromatographic system appears to be efficient for the determination of DMZ, MNZ, and RNZ in meat. The limits of quantification were 0.5, 1 and 5 ng/g, and the detection limits 0.2, 0.4, and 2.5 ng/g for DMZ, MNZ, and RNZ, respectively. The detection limit of the assay was calculated to be three times the baseline noise from drug-free muscle. No interference was seen during analysis, when calibrating the curves, or when performing recovery studies.

In many laboratories, a stream of nitrogen is used to evaporate DNZ, MNZ, and RNZ samples to dryness. We compared nitrogen against air produced from a central air compressor for evaporating the samples of DMZ, MNZ, and RNZ from meat. No differences were observed and air is much cheaper than nitrogen.

Table 1. Recovery and Repeatability for Dimetridazole, Metronidazole, and Ronidazole from Spiked Samples of Pig Muscle

	DMZ		MNZ		RNZ	
Added[a]	5	30	5	30	5	30
No.[b]	8	8	8	8	8	8
Meat						
S.D.%[c]	1.6	2.4	2.2	2.2	1.2	1.8
Rec.%[d]	85	83	85	84	83	87

[a]Concentration ng/g in meat. [b]No. of samples. [c]Standard deviation. [d]Recovery.

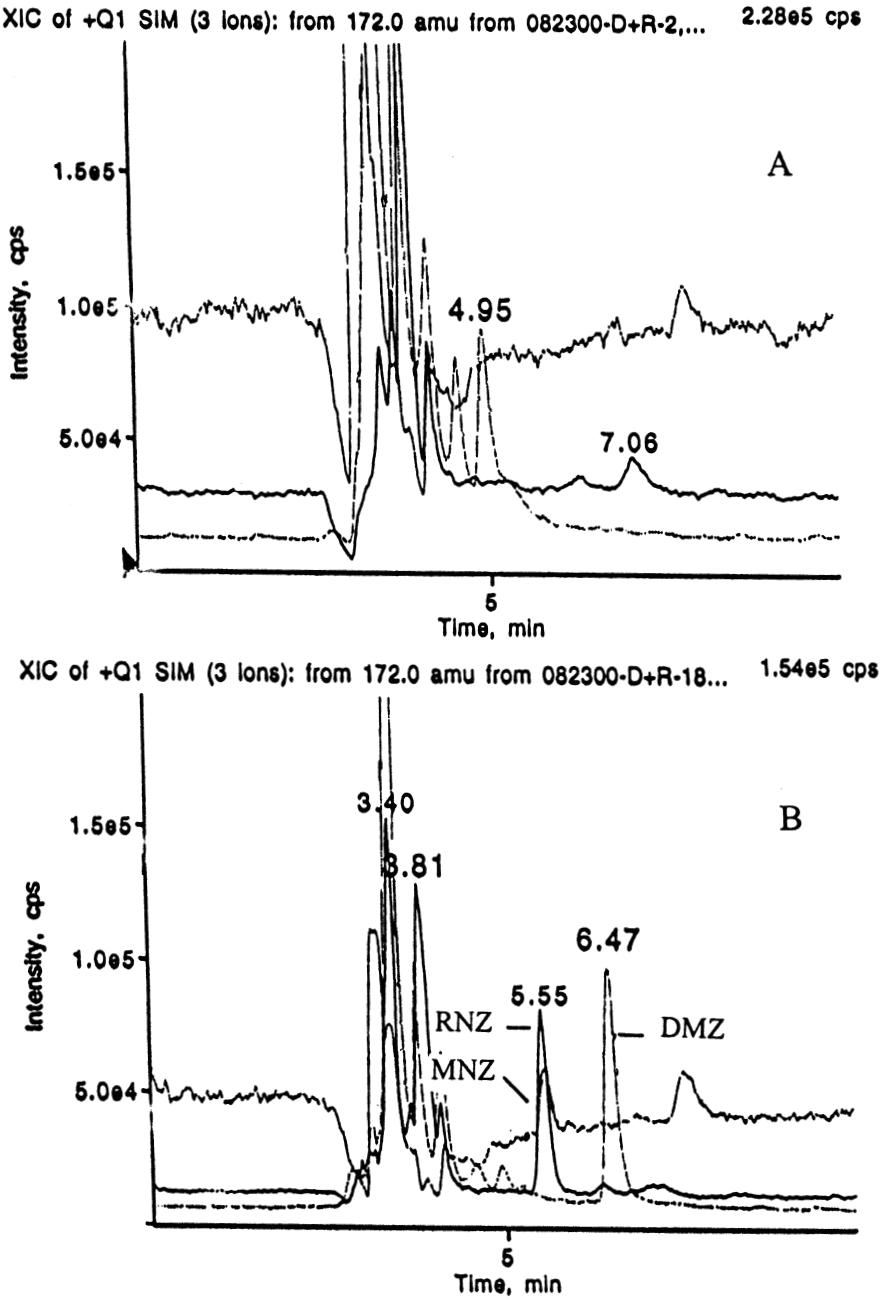

Figure 1. Chromatograms of extracts from pig meat. A: drug-free meat, B: meat spiked with DMZ, MNZ, and RNZ (10 ng/g).

The final evaporation step was identified as critical for good and stable recovery. Not allowing the temperature of the heating block to exceed 40°C also helped to improve the recovery. Both MNZ and RNZ have an almost equal retention time.

Chromatograms of drug-free and spiked meat samples are shown in Figure 1. Chicken muscle extracts show a near similar baseline resolution to samples from pig meat.

The detection limit of the assay depends mainly on the sensitivity of the LC/MS. This in turn could be influenced by such factors as the position of the ion spray inlet, the composition of the mobile phase, and the flow-rate of the mobile phase into the ion source.

The conformity of the graduation of the glass centrifuge tube to the end volume of the sample should be checked beforehand.

In summary, the method described is specific and robust. It has been demonstrated to be efficient for screening and quantification of residues of DMZ, MNZ, and RNZ in muscle.

ACKNOWLEDGMENT

We are grateful to the Norwegian Food Control Authority for financial support.

REFERENCES

1. Council Regulation 2377/90, Off. J. Eur. Commun., 1990, No. L224, 1; modified by Council Regulation 1568/98.
2. Commision Regulation 1798, 25 July 1995. Off. J. Eur. Commun., 1995, No. L174, 20.
3. Council Directive 70/524/EEC., 23 November 1970. Off. J. Eur. Commun., 1970, No. L270, 1.
4. Aerts, R.; Egberink, I.; Kan, C.; Keukens, H.; Beek, W. J. Assoc. Off. Anal. Chem. **1991**, *74*, (1).
5. Semeniuk, S.; Posyniak, A.; Niedzielska, J.; Zmudzki, J. Biom. Chrom. **1995**, *9*, 238-242.
6. Gaugain, M.; Abjean, J. J. of Chrom. A **1996**, *737* 343-346.
7. Sams, M.; Strutt, P.; Barnes, K.; Damant, A.; Rose, M. Analyst **1998**, *123*, 2545-2549.

Received February 10, 2001 Manuscript 5533
Accepted March 22, 2001