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# Ultra performance liquid chromatography–tandem mass spectrometry for the determination of 5-nitroimidazoles and their metabolites in egg

**Invited Paper**

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**Abstract:** An ultra performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS) procedure was developed for the determination of five 5-nitroimidazoles (dimetridazole, ipronidazole, metronidazole, ronidazole and ternidazole) and three of their metabolites (1-methyl-2-hydroxymethyl-5-nitroimidazole, 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and 1-methyl-2-(2'-hydroxyisopropyl)-5-nitroimidazole) in egg matrices. Conditions for UPLC separation and electrospray ionization MS/MS in the positive ion mode were optimized. Samples were prepared by liquid-liquid extraction with buffered aqueous 2.5% trichloroacetic acid followed by solid-phase extraction on a Strata-X-C cartridge with reversed-phase and cation-exchange functionalities. The method's performance was evaluated in accordance with Commission Decision 2002/657/EC, applying the alternative matrix-comprehensive in-house validation approach using specially designed InterVal software. The method was robust against different sample matrix and SPE cartridges, operator change, and changes in sample extract storage. Acceptable apparent recoveries (76 to 109%) were obtained for all analytes. The decision limits ($CC_\alpha$) and detection capabilities ($CC_\beta$) were within the ranges of 0.19 – 2.62 and 0.26 – 4.29 $\mu$g kg$^{-1}$. For all compounds the calibration curve linearity was good with correlation coefficients greater than 0.99. Fifteen eggs and ten whole egg powder samples obtained commercially in Lithuania were analyzed by UPLC–MS/MS; none were found contaminated by 5-nitroimidazoles or their metabolites.

**Keywords:** *UPLC, tandem mass spectrometry • 5-nitroimidazoles • Metabolites • Egg*



## 1. Introduction

Dimetridazole (DMZ, 1,2-dimethyl-5-nitroimidazole), ipronidazole (IPZ, 1-methyl-2-isopropyl-5-nitroimidazole), metronidazole (MNZ, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole), ronidazole (RNZ, 1-methyl-2-(carbamoyloxymethyl)-5-nitroimidazole) and ternidazole (TNZ,1-(3-hydroxyisopropyl)-2-methyl-5-nitroimidazole) are 5-nitroimidazole-based veterinary drugs with antibiotic and anticoccidial activities. The 5-nitroimidazoles are known to be rapidly metabolized, mainly by oxidation of the imidazole ring side chain at C2. For DMZ and RNZ, the major metabolite is 1-methyl-2-hydroxymethyl-5-nitroimidazole (MHMNI), MNZ gives 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (MNZOH) and IPZ gives 1-methyl-2-(2'-hydroxyisopropyl)-5-nitroimidazole (IPZOH). Due to their potentially harmful effects on human health [1,2] DMZ, MNZ and RNZ are listed in Annex IV of Council Regulation 2377/90/EC, and are thus banned from use in food producing animals within the European Union [3] and other countries. IPZ and TNZ have never been authorized as veterinary drugs and are treated as banned substances. Controls concerning observation of these regulations are defined in the EU member states' national residue control plans; efficient test methods are the topics with most emphasis.

Nitroimidazoles and their metabolites in food and other biological matrices may be determined by

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a number of methods [4–10]. However, the confirmation of suspect positive samples must be carried out by mass spectrometry (MS) coupled to adequate chromatographic separation. Although gas chromatography (GC) coupled to mass spectrometry is both sensitive and selective, derivatization of the non-volatile and thermally labile nitroimidazoles is required prior to analysis [5,6]. This procedure significantly increases the overall analysis time and may lead to errors.

Currently, the main improvements in analysis sensitivity and specifity for pharmaceutical residues in foods are by high performance liquid chromatography with tandem mass spectrometric detection (HPLC–MS/MS). This more sophisticated technique allows a very effective isolation of analyte ions from the noise-producing matrix. The potential of HPLC–MS/MS has already been demonstrated for analysis of nitroimidazole residues in complex food matrices such as meat [11–13], egg [11,14] and pig plasma [15]. However, the chromatography is responsible for some limitations. Multi-component analysis of complex samples often requires separation times of 20 min or more. In addition, HPLC–MS/MS has not been used for the simultaneous determination of the five main compounds in this study, along with their metabolites.

Recent advances in analytical instrumentation have led to ultra performance liquid chromatography (UPLC). A high analysis speed, greater resolution, higher peak capacity and improved sensitivity are obtained from a new generation of LC columns packed with pressure-stable 1.7 µm hybrid material particles, along with novel low dead volume, high pressure (1000 bar) equipment [16,17]. As efficiency and analysis speed is very important, UPLC could prove significant in the future of liquid chromatography.

The present work describes a method for the determination of five 5-nitroimidazoles and three of their metabolites (Fig. 1) in egg matrices employing UPLC-MS/MS. Extraction and sample clean-up procedures, as well as UPLC separation and MS/MS detection parameters were optimized. Finally, method validation was performed applying the alternative matrix-comprehensive in-house validation approach using specially designed InterVal (QuoData GmbH, Dresden, Germany) software. This software is a practical application of the statistical approach, which offers a quick and user-friendly determination of all relevant performance characteristics required by Commission Decision 2002/657/EC [18]. InterVal allows the automatic calculation of validation parameters like decision limit ($CC_\alpha$), detection capability ($CC_\beta$), precision (repeatability and within-laboratory reproducibility), % recovery, calibration curves, and prediction intervals.

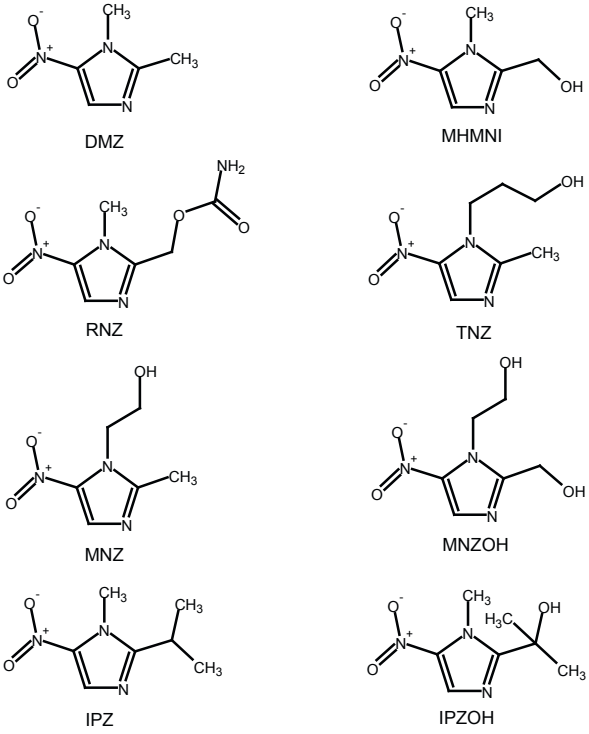


**Figure 1.** Structures of 5-nitroimidazoles and metabolites.

Additionally InterVal delivers details on the measurement uncertainty and the influence of individual factors, *i.e.* it provides comprehensive information on the method's robustness [19–21].

## 2. Experimental Procedures

### 2.1. UPLC–MS/MS instrumentation and conditions

UPLC analyses were performed on a Waters Acquity Ultra Performance LC system (Waters, Milford MA, US using an Acquity BEH C18 column (100 mm × 2.1 mm *i.d.*, 1.7 µm particle size, Waters) at 40°C, with a mobile phase flow rate of 0.6 mL $min^{-1}$. Gradient separation used water acidified with 0.05% formic acid (mobile phase A) and acetonitrile containing 0.05% formic acid (mobile phase B). The gradient was: 0 – 1.0 min 100% A, 1.0 – 7.0 min 75% A, 7.0 – 8.0 min 100% A, and 8.0 – 12.0 min 100% A. The injection volume was 20 µL.

Detection was performed by Quattro Premier XE tandem quadrupole mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization source operating in positive mode. The ionization source parameters were: capillary voltage 2.5 kV, source temperature 120°C, and desolvation gas temperature 350°C. Argon was the collision gas and nitrogen the desolvation gas (800 L $h^{-1}$).

The best multiple reaction monitoring (MRM) transitions and associated acquisition parameters were determined by injecting a standard solution for each analyte. Two transitions per analyte were monitored, while only one was monitored for each deuterated internal standard. The most intense fragment from each precursor ion was selected for quantitation and a less sensitive secondary fragmentation was used for confirmation. Dwell times were set so that approximately 15 data points were acquired for each chromatographic peak (Table 1). Three acquisition windows were set during the analysis, one (1.2 – 2.9 min) for MNZOH, a second (2.9 – 4.6 min) for DMZ, MHMNI, MNZ, TNZ and RNZ, and the third (4.6 – 7.0 min) for IPZ and IPZOH. Data acquisition and processing were performed using MassLynx 4.0 software with the TargetLynx program.

## 2.2. Reagents

Water was purified by a Milli-Q apparatus (Millipore, Bedford, MA, USA). All organic solvents were of HPLC gradient grade and purchased from Sigma–Aldrich (St. Louis, MO, USA). All 5-nitroimidazole analytical standards, including deuterated compounds, were kindly provided by the CRL (BVL, Berlin, Germany). Since deuterated TNZ and MNZOH were not available, these analytes were quantitated using MNZ-$d_3$ as internal standard, due to its structural similarity to TNZ and MNZOH. All other chemicals were of analytical grade and obtained from Sigma–Aldrich. Stock solutions of nitroimidazoles and internal standards were prepared in methanol at 1 mg mL$^{-1}$ and stored at -20ºC, protected from light. Working solutions for spiking blanks were obtained by appropriate dilution.

## 2.3. Procedures

Nitroimidazoles are very light sensitive, therefore all sample preparation procedures were performed in amber-colored vials under yellow light. The optimized sample extraction and lipid removal procedure was as follows: a well-homogenized portion (5 g) of egg was weighed into a 50 mL polypropylene tube, fortified with 20 µL of internal standards (IS) solution (containing 0.1 µg mL$^{-1}$ IPZ-$d_3$, IPZOH-$d_3$ and MNZ-$d_3$, 0.5 µg mL$^{-1}$ HMMNI-$d_3$ and RNZ-$d_3$, and 1.0 µg mL$^{-1}$ DMZ-$d_3$), mixed, and allowed to stand for 5 min in darkness. Then 10 mL 0.2 M sodium acetate buffer (pH 5) and 10 mL of 5% trichloroacetic acid (TCA) were added, the mixture was shaken for 10 min and then centrifuged for 10 min at 3500 *g*. The supernatant was collected in a second 50 mL polypropylene tube and the residue was additionally extracted with 5 mL of 5% TCA. The supernatants were combined and adjusted to pH 5 with 0.5 mL of 5 M NaOH. Strata-X-C cartridge (200 mg / 6 mL, Phenomenex) was preconditioned sequentially with methanol (5 mL), water (5 mL) and 0.2 M acetate buffer (5 mL) and the combined

**Table 1.** Monitored MRM transitions, MS operating parameters, retention times and peak area ratios of 5-nitroimidazoles, their metabolites an deuterated internal standards.

| Analyte | Transition reactions (*m/z*)[a] | Dwell time (s) | Cone voltage (V) | Collision energy (eV) | Retention time (min) | Peak area ratio ± limit (%) |
|---|---|---|---|---|---|---|
| MNZOH | **188→144** | 0.45 | 22 | 13 | 2.65 | 0.98 ± 20 |
| | 188→68 | | | 21 | | |
| MHMNI | **158→140** | 0.20 | 23 | 12 | 3.03 | 0.62 ± 20 |
| | 158→55 | | | 17 | | |
| TNZ | **186→128** | 0.02 | 26 | 15 | 3.09 | 0.52 ± 20 |
| | 186→82 | | | 26 | | |
| MNZ | **172→128** | 0.02 | 24 | 14 | 3.29 | 0.59 ± 20 |
| | 172→82 | | | 24 | | |
| DMZ | **142→96** | 0.02 | 28 | 15 | 3.53 | 0.12 ± 30 |
| | 142→54 | | | 26 | | |
| RNZ | **201→55** | 0.02 | 16 | 21 | 3.54 | 0.45 ± 25 |
| | 201→140 | | | 12 | | |
| IPZOH | **186→168** | 0.08 | 24 | 14 | 5.28 | 0.16 ± 30 |
| | 186→122 | | | 25 | | |
| IPZ | **170→124** | 0.08 | 31 | 17 | 6.46 | 0.69 ± 20 |
| | 170→109 | | | 24 | | |
| MHMNI-$d_3$ | 161→143 | 0.02 | 23 | 13 | 3.03 | |
| MNZ-$d_3$ | 175→131 | 0.02 | 24 | 14 | 3.29 | |
| DMZ-$d_3$ | 145→99 | 0.02 | 28 | 16 | 3.53 | |
| RNZ-$d_3$ | 204→143 | 0.02 | 18 | 12 | 3.53 | |
| IPZOH-$d_3$ | 189→171 | 0.08 | 22 | 13 | 5.25 | |
| IPZ-$d_3$ | 173→112 | 0.08 | 32 | 25 | 6.42 | |

[a] *Transitions in bold are used for quantitation*

supernatants loaded. The cartridge was then rinsed with 5 mL of 5% TCA, 3 mL of methanol and then dried under vacuum. The analytes were finally eluted with 7 mL water/triethylamine/ethylacetate (2.5/2.5/95, v/v/v), which was evaporated under a stream of nitrogen at 35°C, reconstituted in 150 µL of mobile phase A, filtered by centrifugation through 0.2 µm nylon membrane filters (Alltex, Deerfield, IL, USA) and analysed.

Whole egg powder samples were analyzed as described but the sample size was 1 g and 4 mL water was added after the IS fortification.

Matrix-matched calibration curves were prepared and used for quantification. Control egg (whole egg powder) samples previously shown to contain no residues were well-homogenized, weighed, fortified with 20 µL of mixed IS solution and with the appropriate volume (0 – 200 µL) of the working solution to produce the six calibration levels for each analyte: 0, 0.2, 0.4, 0.8, 1.2 and 2.0 µg $kg^{-1}$ for IPZ, IPZOH, MNZ and TNZ; 0, 1.0, 2.0, 4.0, 6.0 and 10 µg $kg^{-1}$ for MHMNI and RNZ; and 0, 2.0, 4.0, 8.0, 12.0 and 20.0 µg $kg^{-1}$ for DMZ and MNZOH. After fortification, samples were prepared as above. Calibration curves were prepared by plotting the response factor (analyte peak area/IS peak area) against analyte concentration.

Analytes were identified by relative retention (ratio of the retention time of the analyte to that of the corresponding IS), and confirmed using the peak area ratio from the two transition reactions according to criteria established in Commission Decision 2002/657/EC [18].

# 3. Results and Discussion

## 3.1. UPLC separation

HPLC separation of nitroimidazoles is usually performed on conventional reversed-phase columns using volatile acidified aqueous-acetonitrile mobile phases [11,14,15]. Acidic additives protonate basic compounds and increase the ESI+ source signal. Initial transfer of the HPLC assay to UPLC was accomplished by using a mobile phase similar to that used in previous papers. Optimal UPLC separation of five nitroimidazoles and three hydroxylated metabolites was achieved using gradient elution with aqueous acetonitrile containing 0.05% formic acid. Elution with the same gradient at different mobile phase flow rates showed that optimal performance, based on compromise among separation time, resolution, and column backpressure, is obtained at 0.6 mL $min^{-1}$. The eight analytes were well-resolved in about 6 min with a total run time of 12 min (including reequilibration).

## 3.2. Extraction and sample clean-up

The 5-nitroimidazole residues are usually extracted from biological matrices with an organic solvent like acetonitrile [11,12], ethyl acetate [10,13,22], or toluene [6] with further cleanup by solid-phase extraction (SPE) [10,11,13,15]. In some cases, the extract is additionally washed with hexane to remove lipids before SPE [10,15]. However, this defatting step reduces recovery of the less polar 5-nitroimidazoles, *e.g.*, IPZ and IPZOH [5,11]. In this study, aqueous TCA solution (20 mL) was chosen as extraction solvent because 5-nitroimidazoles partition well into the acidic aqueous phase. Testing TCA concentrations from 0 to 5% at 1% increments showed no significant improvement in extraction efficiency above 1% TCA.

The effect of repeated extractions was tested by performing second and third extractions with 5 mL of 5% TCA. Two successive extractions with 20 and 5 mL of acidified water provided satisfactory extraction yields for all analytes. No further improvement was obtained either by using more extractions or by altering the volumes of extractants.

The extract from a biological sample contains many compounds in addition to the target analytes. To remove these interferents, a number of clean-up methods have been developed. Most employ at least one solid-phase extraction step with reversed-phase [10,11,23], silica [24,25] or strong cation-exchange sorbent materials [26]. In this study, a mixed-mode polymeric sorbent with reversed-phase and cation-exchange functionalities, Strata-X-C (Phenomenex), was tested for the clean-up of egg extracts.

Aliquots of spiked blank egg extracts were processed through all steps of the SPE to monitor recoveries. We found no significant influence of the aqueous extract pH (from 1 to 6) on extraction yields. However, at lower pH values significantly higher background noise and poorer precision were observed. Neutralizing to about pH 5 produced the cleanest extracts. Buffering was accomplished by the addition of acetate buffer to the aqueous TCA prior to extraction. Consequently, in order to keep adequate acidity for the extraction the TCA concentration was increased to 5%. During subsequent washes with acidified water and methanol no analyte loss was recorded.

Elution of these basic compounds from cation-exchange sorbents is usually by an alkaline solution which deprotonates them and breaks the ionic interaction. Comparison of water/triethylamine/acetonitrile and water/triethylamine/ethyl acetate (each 2.5/2.5/95, v/v/v) mixtures gave higher extraction yields when water/triethylamine/ethyl acetate mixture was the eluent.

**Table 2.** Factors and factor levels used in the validation study.

| Factor | Factor level | | |
|---|---|---|---|
| Matrix | White egg | Brown egg | Whole egg powder |
| Operator | A | | B |
| Storage of extracts | 0 days | | 1 day at 4°C |
| SPE cartridge | Strata-X-C | | Oasis MCX |
| Evaporation temperature | 35°C | | 45°C |

**Table 3.** Spike levels for each analyte used in the validation study.

| Analyte | Concentration, μg kg$^{-1}$ | | | |
|---|---|---|---|---|
| MNZOH | 1.0 | 5.0 | 10 | 20 |
| MHMNI | 0.5 | 2.5 | 5.0 | 10 |
| TNZ | 0.1 | 0.5 | 1.0 | 2.0 |
| MNZ | 0.1 | 0.5 | 1.0 | 2.0 |
| DMZ | 1.0 | 5.0 | 10 | 20 |
| RNZ | 0.5 | 2.5 | 5.0 | 10 |
| IPZOH | 0.1 | 0.5 | 1.0 | 2.0 |
| IPZ | 0.1 | 0.5 | 1.0 | 2.0 |

Finally, to establish the volume of elution solvent required, blank extracts spiked at two concentration levels (2 and 10 μg kg$^{-1}$) were investigated. During SPE, 1 mL fractions were collected, evaporated to dryness, re-dissolved with 150 μL of mobile phase A, filtered and analyzed. At both concentration levels, the total solvent volume required to extract ≥ 60% of the analytes was about 6 mL. To ensure robustness of the method, we used 7 mL in further method development.

## 3.3. Analytical performance

In-house validation was performed in accordance with Commission Decision 2002/657/EC applying the alternative validation approach and using specially designed InterVal software. It is based on a fractional factorial design within a matrix-comprehensive in-house validation. The plan requires the establishment of a leading factor, which can be varied at any number of levels, and up to seven secondary factors varying at two levels. The factors and levels selected for study are presented in Table 2. The sample matrix was defined as the leading factor. As secondary factors which might influence the analysis, the operator, the SPE cartridge, the storage period of the extracts, and the extract evaporation temperature were chosen [27]. Four concentration levels for each analyte were used in the validation study (Table 3). The experiment was divided into 24 batches, resulting in 120 measurements.

Matrix effects were evaluated by building matrix-matched calibration curves and comparing their slopes with those of pure solvent-based curves. However, a complete compensation of both losses during sample preparation and matrix effects was not obtained for either TNZ or MNZOH using MNZ-$d_3$ as internal standard. For reliable quantitation of these analytes a matrix-matched calibration together with the IS should be used. Consequently, this approach was adopted in this work.

Commission Decision 2002/657/EC defines the decision limit ($CC_\alpha$) as the experimental value at or above which a sample is noncompliant (with error probability α). The $CC_\alpha$ for each analyte was established as the concentration at the *y*-intercept of the calibration curve plus 2.33 times its within-laboratory standard deviation. The detection capability ($CC_\beta$) is the smallest analyte concentration that may be detected, identified, and quantified with an error probability of β. The $CC_\beta$ were calculated as the decision limits plus 1.64 times the corresponding standard deviations when analysing blanks spiked at the $CC_\alpha$ level. The decision limits and detection capabilities are summarized in Table 4. Coefficients of variation (CV) of repeatability, within-laboratory reproducibility, and apparent recoveries measured at a second spike level are also given in Table 4. These parameters were also evaluated for other spike levels (results not shown). Apparent recoveries obtained for all analytes in the validated concentration

**Table 4.** Decision limits ($CC_\alpha$), detection capabilities ($CC_\beta$), repeatabilities ($CV_r$), within-laboratory reproducibilities ($CV_{wR}$) and apparent recoveries of the analytes (n = 24)

| Analyte | $CC_\alpha$, μg kg$^{-1}$ | $CC_\beta$, μg kg$^{-1}$ | $CV_r$, %[a] | $CV_{wR}$, %[a] | Recovery, %[a] |
|---|---|---|---|---|---|
| MNZOH | 2.62 | 4.29 | 15.3 | 20.9 | 100.6 |
| MHMNI | 1.25 | 1.77 | 11.5 | 11.8 | 95.2 |
| TNZ | 0.22 | 0.32 | 11.6 | 13.2 | 94.6 |
| MNZ | 0.30 | 0.44 | 16.3 | 17.3 | 98.7 |
| DMZ | 2.37 | 3.39 | 11.8 | 11.9 | 100.6 |
| RNZ | 1.03 | 1.42 | 9.1 | 11.2 | 96.9 |
| IPZOH | 0.21 | 0.30 | 10.3 | 11.1 | 93.0 |
| IPZ | 0.19 | 0.26 | 8.4 | 9.0 | 96.2 |

[a] *Measured at a second spike level (see Table 3)*

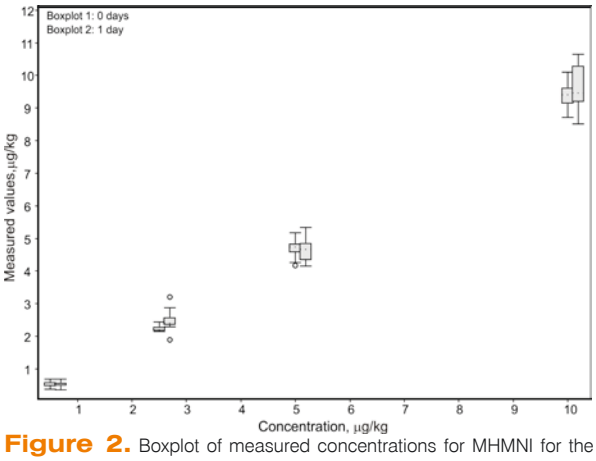


**Figure 2.** Boxplot of measured concentrations for MHMNI for the factor *Storage of extracts*.

range were 76 – 109% and fulfil EU Commission requirements. For most analytes the recovery rate was almost constant over the whole concentration range, while the $CV_r$ and the $CV_{wR}$ values were smaller for higher concentrations than for lower ones.

InterVal shows the overall calibration curves with the prediction intervals as well as the calibration curves for each factor. Factors influencing the calibration curves will show a significantly deviating slope. None of the factors examined showed any significant effect. The linearity of the overall calibration curves was good for all compounds with correlation coefficients always greater than 0.99.

In principle, each factor may be presented individually as a boxplot. As an example, a boxplot of measured concentrations for MHMNI for the factor *Storage of extracts* is given in Fig. 2. The range of the "box" (25 - 75%-quartile) and the range covered by the minimum and maximum (whiskers) at higher MHMNI concentration levels are larger for the factor level *1 day* than for *0 days*. This indicates a greater scatter for sample extracts that have been stored for a day. However, the *t*-value shows that this is not statistically significant.

Finally, fifteen eggs and ten whole egg powder samples obtained commercially in Lithuania were analyzed by the UPLC–MS/MS method and none were found contaminated by 5-nitroimidazoles or their metabolites. For example, Fig. 3 shows the MRM UPLC–MS/MS chromatograms for (a) blank egg sample and (b) egg sample spiked at a concentration level close to $CC_\alpha$.

## 4. Conclusions

The UPLC-MS/MS method described here allows simultaneous determination of five 5-nitroimidazoles and three of their metabolites in egg matrices. The technique provides enhanced resolution and speed of analysis. Separation of the eight compounds was obtained in less than 7 min with a total run time of 12 min, providing the high throughput required for routine analyses. The method was validated according to EU criteria applying the alternative validation approach, and the results showed that the method is accurate, reproducible and sensitive enough.

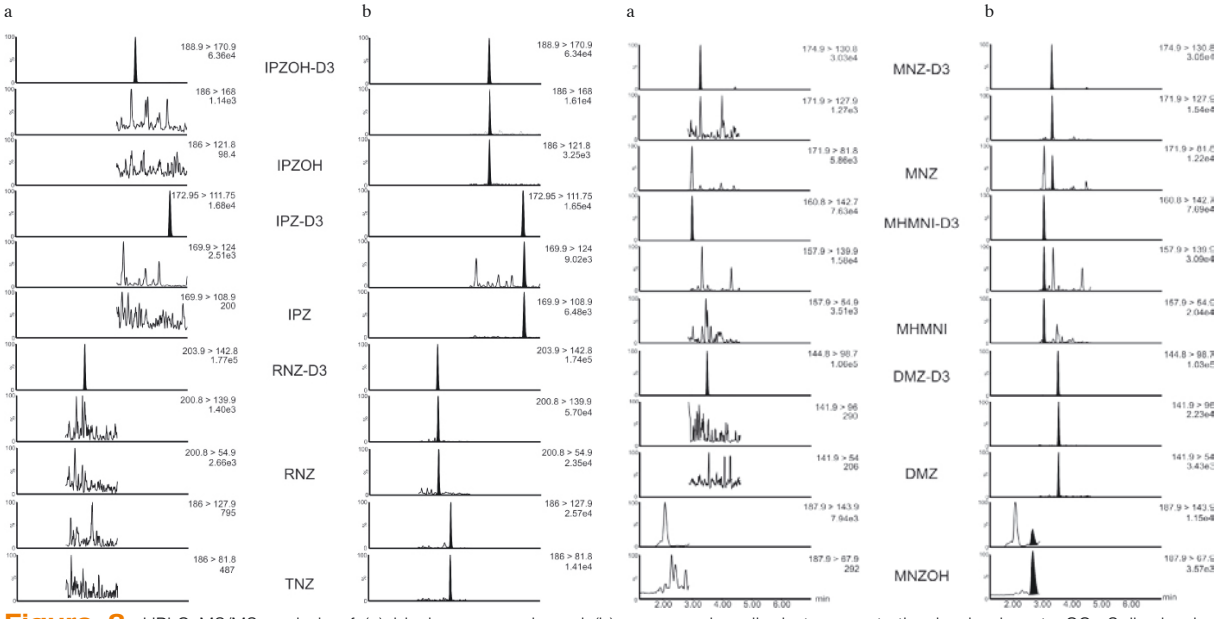


**Figure 3.** UPLC–MS/MS analysis of (a) blank egg sample and (b) egg sample spiked at concentration levels close to CC. Spike levels: 0.22 $\mu$g kg$^{-1}$ for IPZ, IPZOH, MNZ and TNZ; 1.1 $\mu$g kg$^{-1}$ for RNZ and MHMNI; 2.2 $\mu$g kg$^{-1}$ for DMZ and MNZOH.

## References


[1] N.T. Crosby, Determination of Veterinary Residues in Food (Ellis Horwood, Chichester, 1991)
[2] I.E. Voogd, Mutat. Res. 86, 247 (1981)
[3] Council Regulation (EEC) No. 2377/90, Off. J. Eur. Commun. L224, 1 (1990)
[4] L.H. Stanker, C. McKeown, B.E. Watkins, M. Vanderlaan, R. Elis, J. Rajan, J. Agric. Food Chem. 41, 1332 (1993)
[5] J. Polzer, P. Gowik, J. Chromatogr. B 761, 47 (2001)
[6] C. Ho, D.W.M. Sin, K.M. Wong, H.P.O. Tang, Anal. Chim. Acta 530, 23 (2005)
[7] G. Carignan, W. Skakum, S. Sved, J. Assoc. Off. Anal. Chem. 71, 1141 (1988)
[8] R.M. Aerts, I.M. Egberink, C.A. Kan, H.J. Keukens, W.M.J. Beek, J. Assoc. Off. Anal. Chem. 74, 46 (1991)
[9] E.T. Mallinson, A.C. Henry, L. Rowe, J. Assoc. Off. Anal. Chem. 77, 790 (1992)
[10] J.Z. Shen, Y. Yhang, S.X. Zhang, S.Y. Ding, X.H. Xiang, J. AOAC Int. 86, 505 (2003)
[11] P. Mottier, I. Huré, E. Gremaud, Ph.A. Guy, J. Agric. Food Chem. 54, 2018 (2006)
[12] X. Xia, X.W. Li, S.X. Zhang, S.Y. Ding, H.Y. Jiang, J. AOAC Int. 89, 94 (2006)
[13] X. Xia, X. Li, S. Zhang, S. Ding, H. Jiang, J. Shen, Anal. Chim. Acta 586, 394 (2007)
[14] R. Mohamed, P. Mottier, L. Treguier, J. Richoz-Payot, E. Yilmaz, J.C. Tabet, Ph.A. Guy, J. Agric. Food Chem. 56, 3500 (2008)
[15] S. Fraselle, V. Derop, J.M. Degroodt, J. Van Loco, Anal. Chim. Acta 586, 383 (2007)
[16] J. Sherma, J.D. Larkin, F.H. Larkin, J. AOAC Int. 88, 63A (2005)
[17] M. Swartz, J. Liquid Chromatogr. Rel. Technol. 28, 1253 (2005)
[18] Commission Decision 2002/657/EC, Off. J. Eur. Commun. No. L221, 8 (2002)
[19] B. Juelicher, P. Gowik, S. Uhlig, Analyst 123, 173 (1998)
[20] S. Uhlig, P. Gowik, W. Radeck, Anal. Chim. Acta 483, 351 (2003)
[21] C. Bock, C. Stachel, P. Gowik, Anal. Chim. Acta 586, 348 (2007)
[22] D. Hurtaud-Pessel, B. Delépine, M. Laurentie, J. Chromatogr. A 882, 89 (2000)
[23] E.T. Mallinson, A.C. Henry, L. Rowe, J. AOAC Int. 75, 790 (1992)
[24] D.R. Newkirk, H.F. Righter, H.J. Schenck, J.L. Okrasinski, C.J. Barnes, J. Assoc. Off. Anal. Chem. 73, 702 (1990)
[25] A. Cannavan, D.G. Kennedy, Analyst 122, 963 (1997)
[26] M.J. Sams, P.R. Strutt, K.A. Barnes, A.P. Damant, M.D. Rose, Analyst 123, 2545 (1998)
[27] V. Tamosiunas, A. Padarauskas, Chromatographia 67, 874 (2008)