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Research Article

Fast multi-residue method for determination of nineteen benzimidimidazoles in meat tissues by liquid chromatography tandem mass spectrometry

Abstract

A fast, sensitive and selective method has been developed for quantitative determination of residues of nineteen benzimidazoles in meat by speeding the productivity of the conventional liquid chromatographs. The analytes are extracted with phosphate buffer and acetonitrile. Extract is purified by Strata X cartridges. Benzimidazoles are separated within 9 min with a conventional liquid chromatograph. The residues are detected by mass spectrometry at Selected Reaction Monitoring SRM. The separation of the compounds is done on a reversed phase RP-PFP column (50×2.1mm) with 2.6μm core-shell particle size at 50°C. The maximal pressure during analysis is 250 bars. The calculated decision limits (CCα) are between Maximum Residue Limits (MRL)+8% MRL and MRL+33% MRLs. Detection Capability (CCβ) values are in the range MRL+13% and MRL+66%. The detection capabilities (CCβ) are between MRL and 8% MRL and MRL+66% for the range of investigated benzimidazoles. The results of three separate assays (n= 3×6) show the mean recovery between 80% and 110%. Linearity of the method, assessed by coefficient of correlation r², is between 0.990 and 0.995. The accuracy at MRL level of benzimidazoles is <10% with precision below 15%, expressed as Relative Standard Deviation (RSD).

Introduction

Benzimidazoles are anti-parasitic agents used against endo parasites and anthelmintics, widely used when raising animals from which food is produced. Benzimidazoles are used as fungicidal agents too [1]. Some representatives of this drug’s class have teratogenic and embryotoxic effects [2]. Large number of metabolites in edible animal tissues can be identified, for example up to and more than 19 possible residues [3]. To ensure consumers the European Union has set Maximum Residue Limits (MRLs) for benzimidazoles and their metabolites in foods with animal origin. These limits range from 50 to 225μg/kg depending on compound and the type of matrixes (muscle, liver, kidney and fat) [4], (Table 1). For most of the benzimidazoles, the marker residue is defined as the sum of the parent drug and/or its major (or most persistent) metabolite. A comprehensive review of properties and methodologies for determination of benzimidazole residues in biological matrices is presented by Danaher et al., [5]. Despite similarities in the molecule structures and mode of action, benzimidazoles show differences in lipophilicity and pKa values. Moreover, the parent (marker) drugs and their metabolites possess quite various properties. This result into difference in their chromatographic behavior and limited number of benzimidazoles can be determined simultaneously.

There are many scientific works related to benzimidazoles analysis in animal tissues, milk and plant tissues. Numbers of reported methods relate to analysis of individual benzimidazoles and their metabolites in food products [6–9]. However, because of the large number of benzimidazoles licensed for use, multi-residue methods are more useful for the practice. They are more attractive and challenging for the analytical experts, too. These methods could provide more complete surveillance for the drugs. Methods for analysis of enlarged number of benzimidazole residues in milk [10–14] and tissues [3,15–18] have been developed also. Methods for determination of 10 up to 14 benzimidazoles, using Liquid Chromatography–tandem mass spectrometry (LC–MS/MS) were published in the last decade [3,19–22]. The separation of the enlarged number of benzimidazoles is realized for 30–40 min. Danaher et al., [23], separate 14 benzimidazoles simultaneously in a run–time of 60
min and gradient elution program using Xterra C18 column. Kinsella et al., [24], develops a LC – MS/MS multi-residue method for simultaneous identification and quantification of 38 residues of the most widely used anthelmintic veterinary drugs, including some benzimidazoles. Lugomer et al., analyzed 18 benzimidazoles in milk for 28 min [21]. Recently several papers present multiresidue screening methods, based on the increased efficiency of Ultra-High Performance Liquid Chromatography (UHPLC) and High Resolution Mass Spectrometry (HRMS) [25–28]. The reliability of the methods using other types of mass spectrometry as TOF HRMS is higher than that of the Low Resolution Mass Spectrometry (LRMS) and Selected Reaction Monitoring (SRM) mode. These techniques are not applicable and suitable for confirmatory purposes. The beneficial effects of UHPLC with SRM–LRMS have been applied for determination and confirmation of residues of 24 benzimidazoles in meat [29], where the samples are analyzed within 60 min by two runs at positive and negative ionization, respectively. UHPLC with sub-2μm particle size columns become as an attractive technique for improving the separation and/or reducing the time of analysis. The increased back pressure – above 500 bars - requires new generation of Liquid Chromatography (UHPLC) equipment. In order to realize high efficiency the equipment must possesses reduced delay and an acceptable back pressures fewer than 250 bars. These results give us an opportunity to use the convenient liquid chromatographs, it can be realized fast separation at 50°C and an acceptable back pressures fewer than 250 bars. These results give us an opportunity to use the convenient liquid chromatographs. So it can be developed fast multi-residue SRM method for confirmation and quantitative determination of an extended range of benzimidazoles instead the expensive UHPLC instrumentation.

Experimental

Standards: The reference standards and their deuterated analogs Hydroxythiabendazol (TBZ-OH), Albendazolsulfonamide (NH2-ALBZ-SO2), Thiabendazol (TBZ), Albendazolsulfon (ALB-SO), Oxfendazol (OFEB), Hydroxymebendazol (OH-MBZ), Aminomebendazol (NH2-MBZ), Oxibendazol (OBZ), Triclabendazol (TKLBZ) and Fenbendazol (FEB) (Table 2) with porous particles (1.7μm) and this one with core – shell particles (2.6μm), it was established that the last ones possess an equal or higher efficiency than one of the columns with sub-2μm porous particles. Applying these columns to analysis of multi compound mixtures of nonsteroidal anti-flamatory drugs, pesticides and benzimidazoles with conventional liquid chromatographs, it can be realized fast separation at 50°C and an acceptable back pressures fewer than 250 bars. These results give us an opportunity to use the convenient liquid chromatographs. So it can be developed fast multi-residue SRM method for confirmation and quantitative determination of an extended range of benzimidazoles instead the expensive UHPLC instrumentation.

Table 1: Summary of recovery, limit of detection, reproducibility, decision limit (CCα) and detection capability (CCβ) for benzimidazoles in meat at their MRLs and the linearity of the quantitative determination.

| Analyte               | MRL (μg/kg) | LOQ (MRL) | Recovery (%) | Reproducibility (%) | CCα (μg/kg) | CCβ (μg/kg) | Linearity (r²) |
|-----------------------|-------------|-----------|--------------|---------------------|-------------|-------------|----------------|
| ABZ 100               | 0.001       | 91.42     | 8.14         | 116.14              | 132.28      | 0.990       |
| ALB-SO 100            | 0.001       | 114.43    | 12.71        | 120.84              | 141.69      | 0.993       |
| ALB-SO2 100           | 0.001       | 84.62     | 3.99         | 106.54              | 113.08      | 0.995       |
| NH2-ALBZ-SO2 100      | 0.001       | 89.88     | 13.87        | 122.74              | 145.48      | 0.991       |
| MBZ 60                | 0.001       | 99.57     | 2.75         | 64.51               | 69.02       | 0.992       |
| NH2-MBZ 60            | 0.001       | 109.26    | 10.54        | 77.29               | 94.58       | 0.995       |
| OH-MBZ 60             | 0.001       | 105.70    | 5.89         | 69.66               | 79.32       | 0.994       |
| FEBZ 50               | 0.001       | 101.47    | 4.50         | 57.37               | 64.74       | 0.994       |
| OFEBZ 50              | 0.001       | 97.14     | 2.16         | 53.55               | 57.08       | 0.992       |
| FEBZ-SO2 50           | 0.001       | 101.12    | 3.24         | 55.31               | 60.62       | 0.994       |
| FLU 50                | 0.001       | 92.15     | 9.34         | 65.32               | 80.64       | 0.992       |
| NH2-FLU 50            | 0.001       | 91.59     | 8.98         | 64.73               | 79.46       | 0.990       |
| OBZ 100               | 0.001       | 99.88     | 14.31        | 123.47              | 146.93      | 0.995       |
| TBZ-OH 100            | 0.001       | 80.35     | 14.40        | 123.62              | 147.24      | 0.995       |
| TBZ 100               | 0.001       | 81.78     | 10.00        | 116.41              | 132.80      | 0.992       |
| TKLBZ 225             | 0.050       | 94.99     | 12.66        | 245.76              | 266.52      | 0.993       |
| TKLBZ-SO 225          | 0.010       | 100.77    | 12.81        | 278.81              | 332.61      | 0.990       |
| TKLBZ-SO2 225         | 0.010       | 108.95    | 12.78        | 300.08              | 375.15      | 0.990       |
| Keto-TKLBZ 225        | 0.010       | 102.54    | 15.38        | 253.50              | 282.00      | 0.994       |

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purity above 99% were purchased from Witega (Berlin, Germany) and Sigma Aldrich (St. Louis, MO, USA).

**Reagents and materials:** HPLC–grade acetonitrile and methanol were acquired from J.T. Backer Chemical Co (Phillipsburg, NJ). Ultra-purified water was prepared by using a Millipore Milli-Q system (Milford, CT, USA.) water purification system. Sodium bicarbonate (NaHCO₃), di-Potasium hydrogen orthophosphate anhydrous (K₂HPO₃), formic acid, Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solid Phase Extraction cartridges (SPE)-Strata™-X 33μm Polymeric Reversed Phase, 200mg/3ml, tubes were purchased from Phenomenex (Inc. United States). Solid phase extraction was performed using Supelco SPE manifold.

**Stock standard solutions of benzimidazoles:** Stock standard solutions of benzimidazoles and their deuterated analogs (1000 μg.mL⁻¹). were prepared by dissolving 10.00mg of each compound in 10ml DMSO.

**Working standard solutions (10μg.mL⁻¹) of benzimidazoles and working standard solutions (10μg.mL⁻¹) of deuterated ones: prepare by dilution of 100 μl from the stock standard solutions of and, respectively, in methanol using 10 ml volumetric flasks.**

**Samples:** Drug free muscles with different types as cattle, pig, sheep and chicken and origin are being used for the experiments. Muscle samples (100g approximately) were homogenised and kept at -20°C.

**Sample preparation:** Samples were prepared according to the procedure of Federal Office of Consumer Protection and Food Safety BVL Berlin [29]. Portions of 2g of homogenised muscles and 10ml phosphate buffer, pH 8, were mixed with vortex for 1 min in 50ml centrifuge tube. 20ml acetonitrile was added, the mixture was shaken for 10 min and centrifuged for 10 min at 4000rpm. The superenatant was decanted, transferred into another 50ml centrifuge tube and then evaporated to dryness under nitrogen at 40°C. The dried extract was dissolved in Table 2: Summary of the retention times, diagnostic ions, and the MS/MS operating conditions for the 19 benzimidazoles and 10 deuterated internal standards.

| Segment | № | Analyte | t_R (min) | Precursor | Product ions | Col. sn. | Internal Standard |
|---------|---|---------|-----------|-----------|--------------|---------|------------------|
| I (+)   | 1 | Hydroxythiabendazol (TBZ-OH) | 1.66 | 218.1* | 191.1,147.1 | 26 | ALB-SO_d3 |
| 2       | Albendazol sulfonamine (NH₂-ALBZ-SO₂) | 2.06 | 240.1* | 189.1,133.1 | 20 | ALBS-O_d3 |
| 3       | Thiabendazol (TBZ) | 2.15 | 202.1* | 175.1,131.1 | 22 | ALB-SO_d3 |
| 4       | Albendazol sulfoxid_d3 (ALB-SO_d3) | 2.59 | 285.1* | 243.1,208.0 | 22 | IS |
| 5       | Albendazol sulfid (ALB-SO) | 2.59 | 282.1* | 240.1,208.2 | 20 | ALB-SO_d3 |
| II (+)  | 6 | Albendazol sulfid (ALB-SO) | 3.11 | 298.1* | 266.1,159.1 | 18 | ALB-SO_d3 |
| 7       | Albendazol sulfon-d3 (ALB-SO_d3) | 3.10 | 301.1* | 266.2,224.1 | 18 | IS |
| 8       | Oxendazol  (OFEB) | 3.53 | 316.1* | 191.1,159.1 | 24 | OFEB_d3 |
| 9       | Oxendazol-d3 (OFEB_d3) | 3.52 | 319.1* | 194.1,159.2 | 20 | IS |
| 10      | Hydroxymebendazol (OH-MBZ) | 3.66 | 298.3* | 266.0,220.0 | 18 | MBZ_d3 |
| 11      | Aminofendazol (NH₂-MBZ) | 4.09 | 238.1* | 133.1,105.1 | 20 | MBZ_d3 |
| III (+) | 12 | Oxibendazol (OBZ) | 4.63 | 250.1* | 218.0,176.0 | 18 | OXI_d7 |
| 13      | Oxibendazol-d7 (OBZ-d7) | 4.64 | 257.1* | 225.3,135.0 | 18 | IS |
| 14      | Fendazol sulfon (FEB-SO₂) | 4.79 | 323.1* | 299.9,159.9 | 14 | FEB-SO₂_d3 |
| 15      | Fendazolsulfon_d3 (FEB-SO₂-d3) | 4.77 | 335.0* | 300.1,159.0 | 18 | IS |
| 16      | Aminofendazol (NH₂-FLU) | 4.83 | 256.1* | 238.1,123.1 | 18 | ABL-SO_d3 |
| 17      | Megendazol (MBZ) | 5.26 | 296.1* | 264.1,105.0 | 14 | MBZ_d3 |
| 18      | Megendazol_d3 (MBZ-d3) | 5.26 | 299.1* | 264.0,77.0 | 14 | IS |
| IV(*)   | 19 | Albendazol  (ABZ) | 5.96 | 266.2* | 234.0,191.0 | 14 | ABZ_d3 |
| 20      | Albendazol-d3 (ABZ-d3) | 5.96 | 269.1* | 234.1,159.1 | 16 | IS |
| 21      | Flubendazol (FLU) | 5.97 | 313.9* | 282.0,123.1 | 12 | FLU_d3 |
| 22      | Flubendazol_d3 (FLU_d3) | 5.97 | 317.1* | 282.1,195.1 | 14 | IS |
| V(*)    | 23 | Fenbendazol (FEB) | 7.35 | 300.1* | 268.0,159.0 | 20 | FEBZ_d3 |
| 24      | Fenbendazol-d3 (FEB_d3) | 7.34 | 303.2* | 268.2,159.9 | 18 | IS |
| VI (*)  | 25 | Ketotriclabendazol (Keto-TKLZ) | 7.95 | 326.9 | 181.9,146.1 | 18 | TCBZ_d3 |
| 26      | Triclabendazolsulfid (TKLBZ-SO) | 8.11 | 372.9* | 357.9,180.9 | 14 | TCBZ_d3 |
| 27      | Triclabendazolsulfid (TKLBZ-SO₂) | 8.34 | 388.9* | 309.9,243.9 | 24 | TCBZ_d3 |
| 28      | Triclabendazol (TKLBZ) | 8.64 | 356.9* | 341.9,212.0 | 20 | TCBZ_d3 |
| 29      | Triclabendazol_d3 (TKLBZ-d3) | 8.63 | 359.9* | 341.9,196.9 | 18 | IS |

* ions for quantitative determination.

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mixture 1 ml 0.1 M KH₂PO₄ and 5 ml 0.5 M NaHCO₃ with vortex for several seconds.

The Strata X cartridge was pre-conditioned with 5 ml methanol and 5 ml water. The extract was loaded on the cartridge, was washed with 3 ml water and the cartridge was dried by air at 600 mbar for 10 min. The analytes were eluted with 5 ml methanol, evaporated to dryness under nitrogen at 40°C, reconstituted in 500 μL 5% of MeOH in water with vortex for 1 min and transferred into Eppendorf vials. Sample of 50 μl was injected into the chromatograph.

**LC–MS–MS system:** The benzimidazole determination is carried out in a LC–MS/MS System TSQ Quantum Discovery MAX (Thermo Electron Corporation). The electrospray ionization–tandem mass spectrometry (ESI–MS/MS) detection of the benzimidazoles is achieved using a triple stage quadrupole instrument. The negative and positive ionization mode is used, and the ions are monitored in the Selecting-Reaction Monitoring (SRM) mode. The ESIMS/MS conditions are the following: spray voltage (-4) kV; sheath gas (N₂, >95%) 50 (arbitrary units); auxiliary gas (N₂, >95%) 10 (arbitrary units); capillary offset/voltage (-5) V, capillary temperature 350°C. The dwell time was 100 ms/transition. Two transitions are followed for identification but only one was used for quantitation (Table 2). In depends on the retention times of the analytes they are grouped in several time segments and for everyone is constructed scan event with its characteristic ions (Table 2). The analytes, which are ionized in a negative mode (TKLBZ, Keto-TKLBZ, TKLBZ-SO, TKLBZ-SO₂ and TKLBZ-d₃), are analyzed in the last segment (Table 2). The LC effluent was diverted from the mass spectrometer for the first 1.5 min, and again at 8.5 min. Instrumental control and data analysis were performed by Qualbrauser application software from Thermo Electron Corporation. The chromatographic column is Kinetex RP–PPF column (50×2.0 mm i.d., 2.6 μm particle sizes, Phenomenex) equipped with the pre-column type Gemini (3.0 mm×2.0 mm i.d., 5 μm particle sizes, Phenomenex).

A gradient elution mode with mobile phases consisting of 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B), and 0.1% formic acid in methanol (C) is used. The flow rate of 0.75 ml/min is applied to separate the analytes at 50°C (Table 3).

**Method validation:** The validation of the method is carried out according rules of Commission Decision 2002/657/EC [35]: Specificity, linearity, recovery, repeatability, accuracy, decision limit (CC₀) and detection capability (CCₐ).

### Results and Discussion

#### Optimization of LC-MS conditions

Many difficulties are arisen during separation of large number of compounds. Despite the long analyses time at the multi-residue methods, considerable number of the analytes can be co-eluted [25–29]. Kinetex RP–PPF column (50×2.2 mm, 2.6 μm) demonstrates a high efficiency to benzimidazoles (Figure 1). Limits of 250 bars are accepted as a medium value of the pressure at which pumps still work without risk of damages. Another approach applied for reducing the back pressure is to decrease the viscosity of the mobile phase increasing the temperature. Carrying out the separation of benzimidazoles at 50°C, which is also a practically acceptable value, the goal can be achieved. Combination of these two factors gives the possibility to realize acceptable separation and effective resolution of the analytes within 9 min at maximal back pressure of 250 bars. Five analytes can’t be fully separated because of mobile phase composition and molecule structure: triclabendazoles needs negative ionization mode. They can be eluted in the end of the run with one change of positive and negative modes in this segment. Despite the low resolution, ignoring it, it can be obtained reliable SRM mass spectra and quantitative data for all analytes. The SRM scan event for every one of the analytes is combined in six segments (Table 2). The peak shapes has correct Gaussian form. By this way the required three identification points, according to 2002/657/EC as confirmatory criteria for Group B substances are exceeded.

With more than thousands runs of standards, fortified samples, samples of routine laboratory practice, the column retains its efficiency and selectivity (Figure 1). The reproducibility of the relative retention times of analytes, assessed by RSD, is below 2.0%. Both, the positive and negative ionization modes (ESI+/ESI−), are used. Triclabendazoles show much higher [M−H]− abundance under a negative (ESI−) mode. To obtain higher sensitivity, ESI and MS/MS parameters, voltage and temperature of capillary, scan width and scan time, Collision Energy (CE), isolation width and pressure of the collision gas are optimized for each benzimidazoles (Table 2). Because 19 benzimidazoles and their 10 internal standards require large number of scan events, the whole scanning time (9 min) is divided into six segments for acquisition of 15 to 20 points of each one of chromatographic peaks. This ensures accuracy of quantitative analysis, because a signal to noise criterion, S/N, ~ 10 and 20 for LOD and LOQ, respectively, is used. So the false positives signals during determination at levels close to limits of detection were minimized (Table 1).

#### Validation study

The method is validated using porcine muscle as a model matrix at the specified MRLs of each one of analytes. While most of benzimidazole compounds are expressed as the sum of the marker metabolites (Table 1), validation is provided applying MRL value for each individual marker residue. This

### Table 3: Gradient and pressure profiles used for separation of benzimidazoles.

| Time (min) | Comp. A(%) | Comp. B(%) | Comp. C(%) | Pressure (bar) |
|-----------|------------|------------|------------|---------------|
| 0.0       | 95         | 0          | 5          | 220           |
| 1.5       | 80         | 10         | 10         | 250           |
| 5.0       | 70         | 15         | 15         | 220           |
| 6.0       | 65         | 20         | 15         | 180           |
| 8.0       | 45         | 40         | 15         | 160           |
| 8.5       | 5          | 90         | 5          | 160           |
| 8.7       | 95         | 0          | 5          | 220           |
| 10.0      | 95         | 0          | 5          | 220           |
Figure 1: Ion chromatograms of porcine muscle fortified at 1xMRLs of benzimidazoles presented by the characteristic ions, used for quantitative determination.

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is necessary because of the variability in proportions of marker residues which might occur in samples. The concentrations of the analytes are corrected with coefficients, which presents their transformation to metabolites [29]:

**Sum of albendazole:**
\[ m\text{ALBZ}=m\text{ALB-}\text{SO} \times 0.943 + m\text{ALB-}\text{SO2} \times 0.892 + \]
\[ m\text{NH2-ALBZ-}\text{SO2} \times 1.109 \]

**Sum of fenbendazole:**
\[ m\text{FEB-}\text{SO2}=m\text{FEB-}\text{SO}+m\text{FEB} \times 1.107 + m\text{FEB} \times 1.051 \]

**Sum of flubendazole:**
\[ m\text{FLU}=m\text{FLU}+m\text{NH2-FLU} \]

**Sum of mebendazole:**
\[ m\text{MBZ}=m\text{MBZ}+m\text{NH2-MBZ-} \times 1.244 + m\text{OH-MBZ} \times 1.014 \]

**Sum of thiabendazole:**
\[ m\text{TBZ}=m\text{TBZ}+m\text{TBZ-OH} \]

**Sum of triclabendazole:**
\[ m\text{Keto-TCBZ}=m\text{Keto-}\text{TKLBZ} + m\text{TKLBZ} \times 0.916 + m\text{TKLBZ-}\text{SO} \times 0.878 + m\text{TKLBZ-}\text{SO2} \times 0.842 \]

**Selectivity:** To establish the selectivity of the method, five non-fortified samples from every one of matrixes - cattle, pig, sheep and chicken muscle - are processed and analysed according procedures 2.5 and 2.6 respectively. No interfering peaks with the characteristic precursor and two daughter ions were observed at the retention times typical of benzimidazoles (Table 2).

**Linearity of the response:** The linearity of the chromatographic response is defined with samples fortified with standards at five calibration points in concentration range of 0.5–5.0 MRL for each of benzimidazoles. The regression coefficients \((r^2)\) are higher than 0.95 (between 0.990 and 0.995) (Table 1).

**Recovery:** The recovery of the method is determined using samples of porcine muscle fortified at 0.5 MRL, 1.0 MRL and 1.5 MRL levels for each one of benzimidazoles. Mean recovery (number of studies \(n=6\)) of the analytes, calculated in three separate assays, is between 80% and 110%. The reproducibility, presented as RSD%, is 15% (Table 1).

**Decision limit CC, and detection capability CC,** Both of these parameters are determined using calibration curve procedure. Here blank samples are being fortified around MRL levels in equidistant steps (0.5 MRL, MRL and 1.5 MRL). The concentration at MRL plus 1.64 times the standard deviation at the MRL level, give CC, value for each one of the analytes. The CC, value is determined as sum of CC\(\alpha\) value +1.64 times the standard deviation (Table 3). Calculated CC, values in the range between MRL+8% MRL and MRL+33% MRL. CC, values are in the range MRL+13% and MRL+66%.

**LOD and LOQ:** To verify Limits of Detection (LOD) and Limits of Quantification (LOQ) samples are fortified with all analytes on 0.100, 0.010 and 0.001 MRL–levels. LOD and LOQ are calculated on the basis of signal to noise ratio S/N=10 for LOD and S/N=20 for LOQ. The higher efficiency of chromatographic column with core-shell particles aids the LOQ for all analytes to be below CC\(\alpha\) and CC\(\beta\) (Table 3). Only LOQ for TKLBZ-\text{SO} and TKLBZ-\text{SO2} are around 0.01 MRLs because the degree of the negative ionization is lower.

**Accuracy:** Eight samples of each type of matrixes (cattle, pig, sheep and chicken) are fortified with all compounds and their metabolites at concentration level of 1.0 MRL. They are analyzed to estimate the accuracy of the method. It is found that the accuracy of the method is between 4 and 10%. The precision is lower than 15%, expressed as RSD%.

The method is applied in National referent laboratory (NRL) routine analytical work. The method is under the scope of accreditation and is useful for control purposes.

**Conclusion**

The work reported the development and validation a fast RP–HPLC/MS–MS method for determination of residues of 19 benzimidazoles in meat within 9 min. Benzimidazoles compounds were successfully separated by HPLC. The matrix effect are eliminated effectively by employing the appropriate SPE cleanup procedure. The method has satisfactory validation characteristics according to Commission Decision 2002/657/EC for all analytes. The method is suitable for food quality and food safety control as confirmatory method.

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