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序

水产食品作为公认的餐桌佳品，人们不但乐于其美味，更见证了它对人们生活和健康的诸多益处。千百年来，人们将“连年有余（鱼）”作为理想的富庶生活，从中即可反映出水产食品在人们生活中的地位。我国拥有丰富的海洋和内陆水产品生产条件，不断发展的渔业为我国广大渔民提供了大量的就业机会，同时也为国家创造了显著的经济效益。当前我国渔业及渔业经济发生了巨大变化，水产品生产总量已连续十多年名列世界首位，水产品出口占据出口农产品首位。

作为海洋大省的浙江，渔业是其传统产业和重点产业之一，在国民经济中占有十分重要的地位。其中，水产品加工由于具有高附加值、高科技含量、高市场占有率、高出口创汇“四高”特点，经济效益和社会效益明显，已连续多年成为浙江省农产品出口的第一大产业，并在全国具有领先地位。

然而，我们必须认识到，虽然我国水产品加工业有了长足的发展，但与发达国家相比，仍存在很多不足，主要体现在基础研究薄弱、加工与综合利用率比较低、加工产品品种少、附加值低、装备落后、标准体系不健全、产品质量不稳定等方面的不足。

为此，在浙江省委、省政府的领导下，浙江省科技厅于2009年批准成立浙江省水产品加工产业创新团队，旨在通过整合浙江省水产品加工的科技与产业资源，加强水产加工基础研究，建设一个集水产加工科技源头创新、成果转化与人才培养为一体的现代水产加工利用和质量安全控制技术创新平台，提高浙江省水产品加工利用的技术创新能力。

本论文集是创新团队成员，尤其是中青年学术骨干围绕各自专长潜心研究多年的最新研究成果，内容涵盖水产品贮藏与加工、水产品质量安全与控制以及水产品营养与功能性水产食品开发。希冀此论文集的问世能够助推我国水产品精深加工领域的科技创新，为我国渔业经济的持续、繁荣发展贡献力量。

中国工程院院士 

2012年8月30日

目　录

Simultaneous Determination of Albendazole and Its Metabolites in Fish Muscle Tissue by Stable Isotope Dilution Ultra-performance Liquid Chromatography Tandem Mass Spectrometry

Xiaojun Zhang[1,2*] Hanxiang Xu[1] Hong Zhang[2**] Yuanming Guo[1]
Zhiyuan Dai[2] Xuechang Chen[1]

1 Introduction

Albendazole ([5-(propylthio)-1*H*-benzimidazol-2yl]carbamic acid methyl ester) is a potent broad-spectrum benzimidazole anthelmintic agent widely used against intestinal helminth infections in mammals[1]. The albendazole(ABZ), after oral treatment, is readily absorbed from the gut and converted into its metabolites, albendazole sulfoxide (ABZSO), albendazole sulfone ($ABZSO_2$) and albendazole 2-aminosulfone (ABZ-2-NH_2SO_2). Figure 1 gives structures of ABZ and its metabolites. Toxicological studies in both farm and laboratory animals have shown ABZ and its active metabolite ABZSO to be teratogenic[2]. ABZ, sometimes used in aquaculture, and the residues of ABZ and its metabolites in fish products may pose health risks to consumers. Because of the differences of lipophilicity and pK_a values among ABZ and its metabolites, the determination of benzimidazole multiresidues in biological matrices is a challenge. Therefore, appropriate analytical methodology is demanded and must provide enough sensitivity and the ability to confirm the identity of ABZ and its metabolites in fish muscle tissue.

Many analytical methods for the determination of ABZ and its metabolite residues in biological matrices are described in the scientific literature. Most of them rely on immunoassay[3], high-performance liquid chromatography (HPLC) with UV[4], HPLC with fluorescence detection[5—9] and high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS)[10—20]. Although HPLC is a well-established technique, it shows relative lower sensitivity and specificity compared to the HPLC-MS/MS method.

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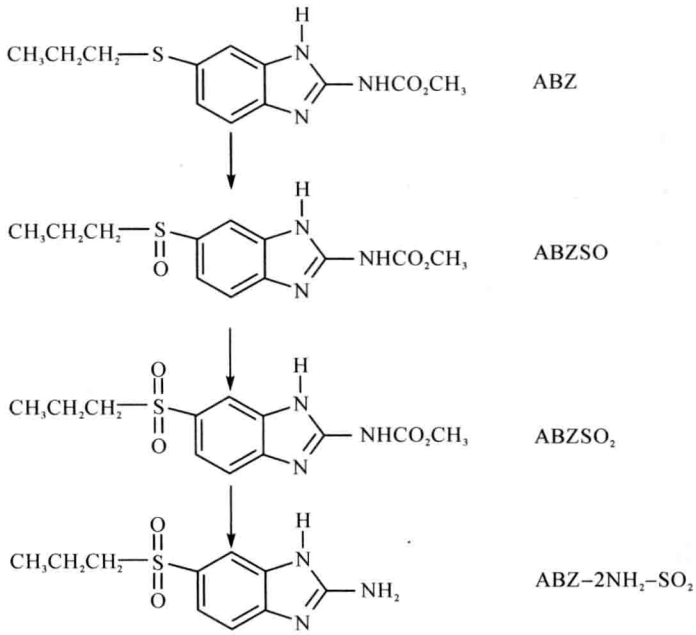

Figure 1 Chemical structures of ABZ, ABZSO, $ABZSO_2$ and $ABZ\text{-}2NH_2\text{-}SO_2$

The LC-MS/MS method significantly enhances the sensitivity and specificity[13], but the chromatographic run times are relatively long time[12] for high-throughput analyses, and sometimes cannot provide satisfied reproducibility. The reproducibility of quantitative LC-MS/MS method usually improves when incorporated with isotopically labelled internal standard. To the best of our knowledge, due to the lack of proper isotopically labeled internal standard, no methods have been described to use isotope dilution for the analysis of ABZ with LC-MS/MS.

This paper describes an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for determination of ABZ and its metabolites in fish muscle tissue using stable isotope-labeled internal standards. The isotope-labeled internal standards were used to correct the matrix effect and variations associated with analysis, and ultra-performance liquid chrpmatography (UPLC) techniques offer efficient chromatographic separation with reduced run times and improved sensitivity. The developed methodology gave good sensitivity, recoveries and reproducibility. It provides a suitable method for the determination and confirmation of ABZ and its metabolite residues in fish muscle tissue and can be used for residue control programs.

2 Experiment

2.1 Chemicals and Reagents

Liquid chromatography (LC)-grade methanol and acetonitrile was purchased from

Merk KGaA (Darmstadt, Germany); LC-grade ethyl acetate and formic acid were purchased from Sigma-Aldrich (Seelze, Germany). ABZ, ABZSO, $ABZSO_2$, ABZ-2-NH_2SO_2, deuterated albendzole (D_3-ABZ), deuterated albendazole sulfone (D_3-ABZSO) and deuterated albendazole sulfone (D_3-$ABZSO_2$) was obtained from Adlershof GmbH (Berlin, Germany; purity≥98%). De-ionised water was used throughout the study.

2.2 Preparation of Standard Solutions

Individual stock standard solutions (100 $\mu g\ mL^{-1}$) were prepared by dissolving 5.0 mg of compound in 50 mL of methanol. Stock internal standard solutions (100 $\mu g\ mL^{-1}$) were prepared in a similar way. Mixed working standard solutions (100 and 10 ng mL^{-1}) were prepared by the dilution of the proper stock solution with methanol. Stock standard solutions were stored at −20℃ and were stable for 6 months. Working standard solutions were stored at 4℃ and were stable for at least 1 month.

2.3 Sample Preparation

The 2.00 ± 0.02 g thawed and homogenized sample was weighted into a 50-mL polypropylene tube and then spiked with 100 μL 100 ng mL^{-1} internal standard working solution. After the addition of 100 μL 10 M sodium hydroxide and 15 mL ethyl acetate, the tube was mixed on a vortex mixer for about 5 min. The sample was then centrifuged for 5 min at 6,000 rpm, and the supernatant was transferred to a 100 mL pear-shaped flask. An additional 15mL ethyl acetate was added to the sample tube, mixed and centrifuged as above. The resulting supernantant was combined and evaporated to dryness using a rotary evaporator (40℃, 0.1 mbar). The residue was then dissolved in 2mL methanol-water solution (40 : 60, *v*/*v*) and ultrasonicated for 1 min. The solution was transferred to a 15-mL tube; 2.0 mL *n*-hexane was added and vortexed for 2 min, then centrifuged for 5 min at 6000 rpm. The 1.0 mL lower aqueous layer was filtered through a 0.22 μm PTFE filter and transferred to a glass vial for UPLC-MS/MS injection.

2.4 Instrumentation

Chromatographic separation was performed on an ACQUITY ultra-performance LC system (Waters, Milford, MA, USA) equipped with cooling autosampler and column oven. The following UPLC columns were investigated for separations: BEH C_{18} 2.1×50 mm, 1.7 μm particle size; BEH C_8 2.1×50 mm, 1.7 μm particle size ; CSH C_{18} 2.1×50 mm, 1.7 μm particle size (Waters, Milford, MA, USA). The column temperature was maintained at 40 ℃. The mobile phase was composed of methanol (A) and aqueous formic acid 0.2% (*v*/*v*) (B) using a gradient elution as follows: 0～2 min, 10%～90% A; 2～3 min, 90% A; 3～4 min, return to initial conditions to equilibrate the column. The flow rate was kept at 0.3 mL min^{-1}. The temperature in the autosampler was set at 10℃ and the sample volume injected was set at 10 μL.

A Quattro Premier XE™ Micromass® triple-quadrupole mass spectrometer (Manchester, UK) equipped with an electrospray ionization (ESI) source was used for ABZ and its metabolites detection. MS/MS detection was performed in the positive mode. The optimal MS parameters were as follows: Source temperature 120℃, desolvation temperature 380 ℃, capillary voltage 3.5 kV, cone gas flow 50 L h^{-1}, desolvation gas flow 600 L h^{-1}. Nitrogen (99.9% purity) and argon (99.9999% purity) were used as cone and collision gases, respectively. Individual cone and collision energy voltages, as well as multiple reaction monitoring mass transitions are summarized in Table 1.

Table 1 Analyte and internal standard transition ions and associated mass spectrometric parameters

Analyte	Precursor ion (m/z)	Product ions (m/z)	Cone voltage (V)	Collision energy (eV)	Internal standard
ABZ	266.0	234.1[a] 191.0	40	20 30	D_3-ABZ
ABZSO	282.0	240.1[a] 222.1	30	15 20	D_3-ABZSO
$ABZSO_2$	298.0	266.0[a] 159.0	40	35 20	D_3-$ABZSO_2$
ABZ-2NH_2-SO_2	240.0	133.1[a] 198.1	30	25 20	D_3-$ABZSO_2$
D_3-ABZ	269.0	234.1[a]	40	20	
D_3-ABZSO	285.0	243.1[a]	40	15	
D_3-$ABZSO_2$	301.0	266.1[a]	40	20	

[a] Multiple reaction monitoring ions used for quantifications.

2.5 Calculations

Data acquisition was acquired and processed using MassLynx V4.1 and QuanLynx software. Calibrators were used for the construction of a standard curve by plotting the response (y=area analyte/area internal standard) against the corresponding concentrations. The concentrations of ABZ and its metabolites in fish muscle tissue were calculated by linear regression. The use of internal standard was automatically calculated.

2.6 Method Validation

2.6.1 *Specificity*

The specificity of the method was determined by analysis blank fish muscle samples from different sources to evaluate possible endogenous interferences. The sample preparation and chromatographic condition were optimized to guarantee that no interferences incurred at the retention times of the tested compounds.

2.6.2 *Linearity*

The linearity of standard calibratin curve was studied by the analysis of standard

solutions at six concentrations (0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 ng g^{-1}). To each sample 100 μL 100 ng mL^{-1} internal standard working solution was added. Linear calibration curve was obtained by least-squares linear regression procedures.

2.6.3 *Precision and Accuracy*

Accuracy was expressed as recovery, calculated by the measured concentration/the fortified level×100%. It was measured using a six determinations at three concentrations (0.2, 1.0 and 5.0 ng g^{-1}), and the standard deviation was calculated. Precision of the method was assessed by determining intra-and-inter-day relative standard deviation (RSD). Both recoveries and RSD of the method were tested with spiked fish muscle samples at three different concentrations. Intra-day RSD were determined by replicate analyses (n=6) performed on the same day. Inter-day RSD were determined by replicate analyses on six different days.

3 Results and Discussion

3.1 Optimization of method

3.1.1 *Selection of Extraction Solvent*

The selection of an appropriate extraction solvent is an important aspect for the sample preparation. Due to considerable variation in lipophilicity and p*K*a among ABZ and its metabolites, a study of extraction behavior was conducted to identify an optimal solvent for extracting target analytes from fish muscle tissue. Three solvents methanol, acetonitrile and ethyl acetate were tested with spiked samples(1.0 ng g^{-1}) in a side-by-side comparison. Furthermore, mean recoveries ($n = 5$) were calculated for individual analytes. Individual analyte recoveries were averaged for each solvent extraction and charted in Figure 2. Results indicate that ethyl acetate has the higher extraction efficiency for the four analytes. Thus, ethyl acetate was selected as the extraction solvent for the sample preparation.

3.1.2 *Selection of Internal Standard*

For a proper internal standard, it should be structurally or chemically similar to the analyte, and should also have similar retention to the analyte, be well resolved from the analyte and other peaks, and mimic the analyte in any sample preparation steps. For the analysis of ABZ and its metabolites, butylmercaptobenzimidazole, estazolam[11], phenacetin[12—13] and dehydroandrographolide[15] were used as internal standards. However, these substances did not perform well, behaving with the low recovery and imperfect accurateness. In this research, deuterated substances were chosen as the internal standard for the essay because of the likeness of structure, retention and ionization to the analyte. D_3-ABZ, D_3-ABZSO and D_3-$ABZSO_2$ were used as the internal standards of ABZ, ABZSO and $ABZSO_2$, respectively. Because of the lack of isotopically labeled internal

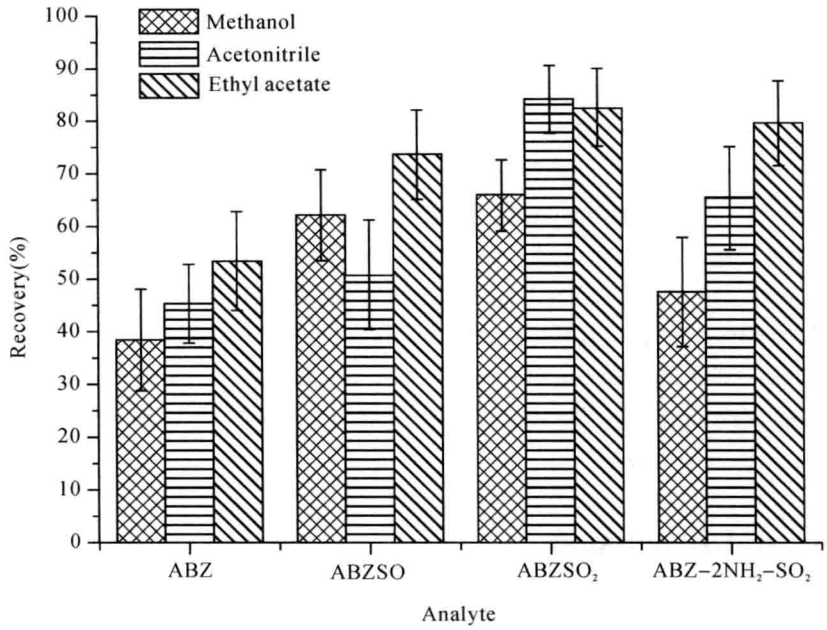

Figure 2 Recoveries for extraction of ABZ and its metabolites from fish muscle tissue using methanol, acetonitrile, and ethyl acetate

standard, D_3-ABZ, D_3-ABZSO and D_3-$ABZSO_2$ were tested to quantify ABZ-2-NH_2SO_2. Results showed that D_3-$ABZSO_2$ that gave the best recovery and low RSD in quantitative analysis could be used as a suitable internal standard of ABZ-2-NH_2SO_2.

3.1.3 *Optimization of UPLC-MS/MS Conditions*

The electrospray ionization interface parameters need to be optimized for maximum abundance of the molecular ions of the compounds. Acquisition parameters were determined by direct infusion into the mass spectrometer of a 1.0 $\mu g\ mL^{-1}$ solution of each one of the analytes and internal standard (IS) at a flow rate of 10 $\mu L\ min^{-1}$. ESI probe temperature was set at the minimum acceptable value (120℃) and capillary voltage was kept at 3.5 kV. Mass spectrum of the analytes and the obtained IS in ESI-positive ion mode and under the optimum conditions of mass spectrometric presented in Figure 3. The fragmentation pathways of ABZ and its Metabolites have been studied in previous publications[11-12], while the isotope-labeled IS were not covered in these studies. For deuterated ABZ and deuterated $ABZSO_2$, the fragment ion at m/z 234 and 266 was formed by loss of a deuterated neutral methanol from $[M+H]^+$ ion. Similarly, they have the same fragment ions with ABZ and $ABZSO_2$ respectively(Figure 3-B and 3-D). While for deuterated ABZSO, the fragment ion at m/z 243 was different to the fragment ion of ABZSO (m/z 240) (Figure 3-E and 3-F). Results indicate that the isotopic fragment ion was formed by loss of $-CH_2\ CH_2\ CH_3$ from $[M+H]^+$ ion, which was the same loss for ABZSO.

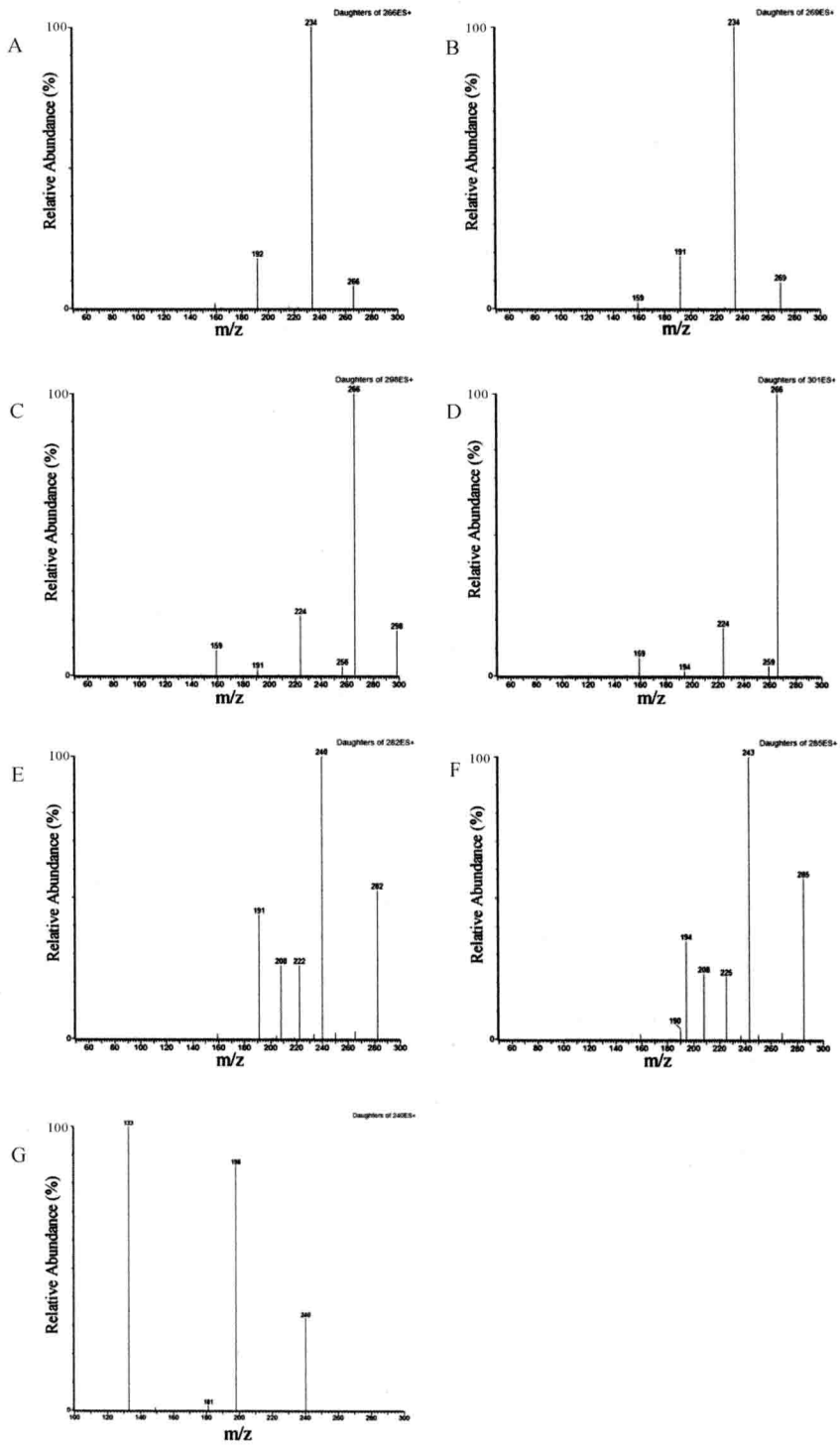

Figure 3 MS/MS fragmentation spectra of analytes and the IS: (A) ABZ; (B) D_3-ABZ; (C) $ABSO_2$; (D) D_3-$ABSO_2$; (E) ABSO; (F) D_3-ABSO; (G) ABZ-2NH_2-SO_2

Chromatographic conditions were optimized to obtain shorter run time and best peak shape. During the method development, it was found that the component of final dissolving solution influenced the peak shape of ABZ-2NH_2-SO_2 significantly. The analytes dissolved in methanol-H_2O (40 : 60, v/v) containing 0.2% formic acid produced good peak shape and sensitivity. To select a proper column, BEH C_{18}, BEH C_8, CSH C_{18} were compared for the separation. The result was summarized briefly below: ABZSO and $ABZSO_2$ give the same retention time on BEH C_8 column; ABZ-2NH_2-SO_2 was not well separated and performed as a double-humped peak on CSH C_{18} column. BEH C_{18} column with a gradient elution gave the best separation for the analytes, and selected as the proper column for Chromatographic separation. After optimization of the gradient profile, the four compounds in the mixture were well-separated from one another within 4 min. Figure 4 shows a typical separation of a standard mixture of ABZ and its metabolites with BEH C_{18} column. The analysis time was faster than the existing method[17].

3.2 Method Validation

3.2.1 *Specificity*

Matrix effects are commonly encountered in LC electrospray mass spectrometry analysis of target compounds in complex sample matrices. To check the specificity of the method, interference from endogenous compounds around the retention times of the analytes was investigated by analysis of three different blank samples(grass carp, tilapia and eel). Result shows that no interferences were observed at the retention times of analytes.

3.2.2 *Linearity and Sensitivity*

For the construction of the calibration curves, the areas of the analyte and the internal standard were calculated, and their ratio was used as the response variable. A calibration curve is constructed by linear curve fitting using the least squares linear regression calculation. Six points are used for the calibration curve of the standard solutions at concentrations of 0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 ng g^{-1} with the internal standard at a concentration of 10.0 ng g^{-1}. The linearity was good for all analytes in the whole range of tested concentrations (0.1～20 ng g^{-1}), as proved by the correlation coefficients (r^2) ranging between 0.9985 and 0.9992 for all curves (Table 2).

The sensitivity was evaluated by determining the limits of detection LOD and limit of quantitation (LOQ). According to the FDA guide on Analytical Procedures and Methods Validation[21], LOQ was defined as the lowest concentration that could be determined with 80%～120% accuracy and not higher than 20% precision value, and the analyte response should be at least 5 times compared to blank response. The LOD was defined as the concentration with a signal-to-noise ratio (S/N) of 3. The background noise estimate was based on the peak-to-peak baseline near the analyte peak. In this research, the peak height-to-averaged background noise ratio was measured by MassLynx V4.1 automatically. The method

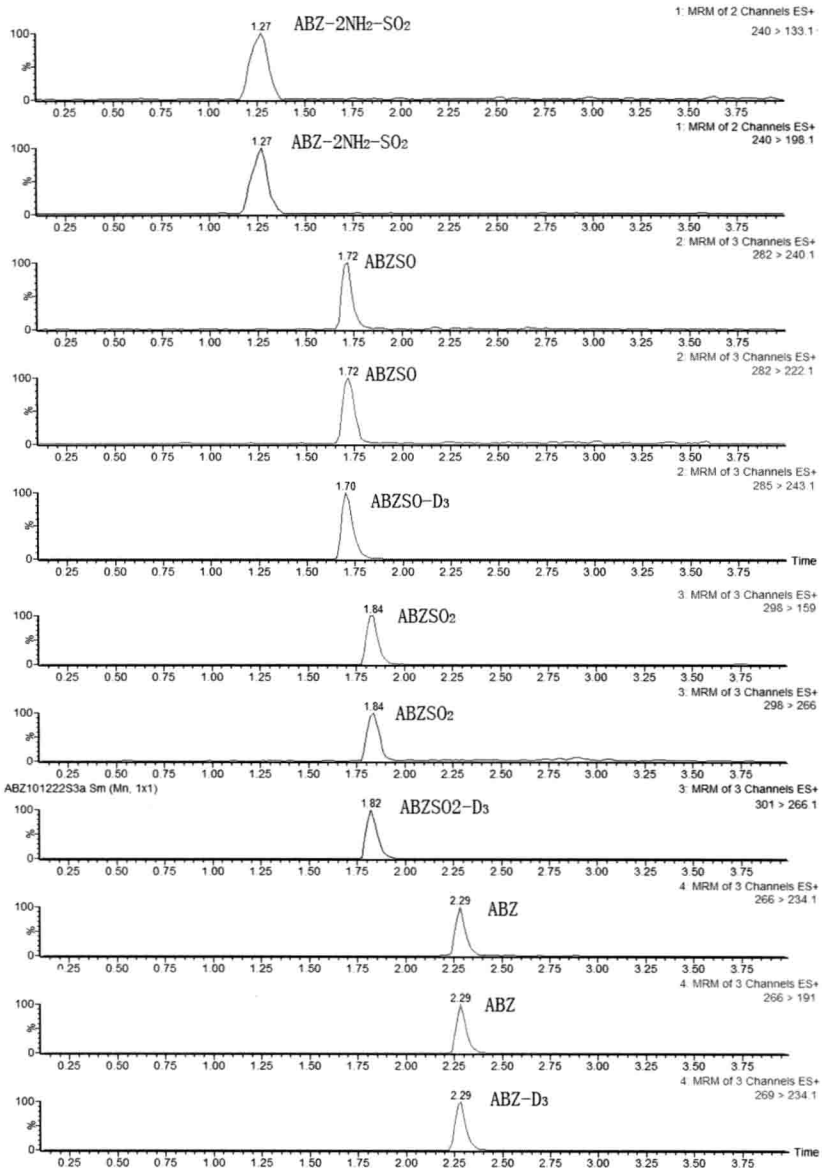

Figure 4 Multiple reaction monitoring chromatogram from a standard mixture of ABZ, ABZSO, $ABZSO_2$, $ABZ\text{-}2NH_2\text{-}SO_2$, and internal standards

LOD were found to be 0.1 ~ 0.2 ng g^{-1}, with the S/N of ABZ, ABZSO, $ABZSO_2$, $ABZNH_2SO_2$ at LOQ were 25.0, 20.8, 23.8, 16.2 respectively. The accuracy and precision also met the requriment mentioned above. The method LOD and LOQ values are summarized in Table 2.

Table 2 Linear range, correlation coefficient, and limits of detection and quantification

Analyte	Linear regression equation	Correlation coefficient, r^2	Liner range (ng mL^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})
ABZ	y=0.4320x+0.0021	0.9991	0.1—20	0.03	0.1
ABZSO	y=0.4071x+0.0179	0.9985	0.1—20	0.03	0.1
$ABZSO_2$	y=0.6303x+0.0234	0.9992	0.1—20	0.03	0.1
ABZ-$2NH_2$-SO_2	y=1.1744x+0.0172	0.9988	0.1—20	0.05	0.2

3.2.3 *Precision and Accuracy*

To improve the accuracy and precision of LC-MS/MS quantitative method, stable isotope-labeled IS was used to compensate the analyte losses during sample preparation and the ion suppression. Recovery was measured using six determinations at concentration of 0.2, 1.0 and 5.0 ng g^{-1}, and then the mean recovery and standard deviation were calculated at per concentration. Intra-day RSD were determined by replicate analyses (n=6) performed on the same day; inter-day RSDs were determined by replicate analyses on six different days. The mean recoveries, intra-day and inter-day RSD of the method are shown in Table 3. Mean recoveries ranged between 97.2% and 113.7% with intra-and inter-day RSD values less than 6.38%, demonstrating the good accuracy and precision of the method.

Table 3 Results of recovery test and precision of the method from spiked fish muscle samples

Analyte	Added (ng g^{-1})	Mean recovery (%, n=6)	Inter-day RSD (%, n=6)	Intra-day RSD (%, n=6)
ABZ	0.2	105.3	3.52	4.25
	1.0	95.3	2.63	3.07
	5.0	99.6	3.18	3.66
ABZSO	0.2	108.2	4.77	4.21
	1.0	109.4	5.14	3.93
	5.0	103.3	4.52	3.38
$ABZSO_2$	0.2	104.2	4.33	4.91
	1.0	97.2	4.21	4.14
	5.0	106.4	3.79	2.59
ABZ-$2NH_2$-SO_2	0.2	105.1	6.38	4.38
	1.0	113.7	4.56	5.03
	5.0	103.8	5.42	3.64

3.3 Real Samples Analysis

The method was applied to 60 grass carp, tilapia and eel tissue samples collected from local markets (Zhoushan, China). All samples were processed according to the method described. The samples were analyzed, and an eel sample was confirmed positive. The concentration of the positive sample was 0.86 ng g^{-1} (ABZ) , 0.34 ng g^{-1} (ABZSO) and 0.27 ng g^{-1} ($ABZSO_2$).

4 Conclusions

This paper describes a rapid, specific, and sensitive UPLC-MS/MS method for simultaneous determination of ABZ, ABZSO, $ABZSO_2$ and ABZ-2-NH_2SO_2 in fish muscle tissue using stable isotope-labeled internal standards. The isotope-labeled internal standards were used to correct the matrix effect and variations associated with the analysis. UPLC technique offers efficient chromatography with reduced run time (4.0 min) and improved sensitivity. This method is suitable for the determination and confirmation of ABZ and its metabolite residues in fish muscle tissue and can be used for residue control programs.

References

[1] MCKELLAR Q A, SCOTT E W. The Benzimidazole Anthelminticsagents-A Review[J]. J Vet Pharmacol Ther, 1990(13):223—247.

[2] DELATOUR P, PARISH R . Benzimidazole Anthelmintics and Related Compounds: Toxicity and Evaluation of Residues[M]. New York: Academic Press, 1986.

[3] BRANDON D L, BINDER R G, BATES A H, et al. Monoclonal Antibody for Multiresidue ELISA of Benzimidazole Anthelmintics in Liver [J]. J Agric Food Chem, 1994(42):1588—1594.

[4] CHIAP P, EVRARD B, BIMAZUBUTE, et al. Determination of MA, Albendazole and Its Main Metabolites in Ovine Plasma By Liquid Chromatography with Dialysis as an Integrated Sample Preparation Technique[J]. J Chromatogr A, 2000(870): 121—134.

[5] NATHAN R, BADAR S. Determination of Albendazole and Its Metabolites in the Mscle Tissue of Hybrid Striped and Large Mouth Bass Using Liquid Chromatography with Fluorescence Detection[J]. J AOAC Int. , 2008(91):469—477.

[6] BADAR S, NATHAN R. Determination of Albendazole and Its Major Metabolites in the Muscle Tissues of Atlantic Salmon, Tilapia, and Rainbow Trout by High Performance Liquid Chromatography with Fluorometric Detection[J]. J Agric Food Chem,2003(51): 3254—3259.

[7] DIMITRIOS J, ELIAS P. Highly Sensitive Ion Pair Liquid Chromatographic Determination of Albendazole Marker Residue in Animal Tissues[J]. J Agric Food Chem, 2005(53): 893—898.

[8] SHAIKH B, RUMMEL N, GIESEKER C, et al. Metabolism and Depletion of Albendazole in the Muscle Tissue of Channel Catfish Following Oral Treatment [J]. J vet Pharmacol Therap, 2006, 29 (6): 525—530.

[9] BATZIAS G C, DELIS G A. Reversed-Phase Liquid Chromatographic Method with Fluorescence

Detection for the Simultaneous Determination of Albendazole Sulphoxide, Albendazole Sulphone and Albendazole 2-Aminosulphone in Sheep Plasma[J]. J Chromatogr B, 2004,805(2):267—274.

[10] MSAGATI T, NINDI M. Comparative Study of Sample Preparation Methods: Supported Liquid Membrane and Solid Phase Extraction in the Determination of Benzimidazole Anthelmintics in Biological Matrices by Liquid Chromatography - Electrospray - Mass Spectrometry[J]. Talanta, 2006(69):243—250.

[11] CHEN X,ZHAO L,XU H, et al. Simultaneous Determination of Albendazole and Its Major Active Metabolite in Human Plasma Using a Sensitive and Specific Liquid Chromatographic - Tandem Mass Spectrometric Method[J]. J Pharm Biomed Anal, 2004(35):829—836.

[12] BONATO P S,OLIVEIRA A R, SANTANA F J, et al. Simultaneous Determination of Albendazole Metabolites, Praziquantel and Its Metabolite in Plasma by High-Performance Liquid Chromatography - Electrospray Mass Spectrometry[J]. J Pharm Biomed Anal,2007(44): 558—563.

[13] BONATO P S, LANCHOTE V L,TAKAYANAGU O M. Simultaneous Liquid Chromatography - Tandem Mass Spectrometric Determination of Albendazole Sulfoxide and Albendazole Sulfone in Plasma[J]. J Chromatogr B, 2003(783):237—245.

[14] YAMADA R, KOZONO M, OHMORI T, et al . Simulaneous Determination of Residual Veterinary Drugs in Bovine Porcine, and Chicken Muscle Using Liquid Chromatography Coupled with Electrospray Ionzation Tandem Mass Sepctrometry[J]. Biosci Biltechnol Biochem, 2006(70): 54—65.

[15] BALIZS G. Determination of Benzimidazole Residues Using Liquid Chromatography and Tandem Mass Spectrometry[J]. J Chromatogr B, 1999(727): 167—177.

[16] HU X, WANG J, FENG Y. Determination of Benzimidazole Residues in Edible Animal Food by Polymer Monolith Microextraction Combined with Liquid Chromatography-Mass Spectrometry[J]. J Agric Food Chem,2010(58):112—119.

[17] XIA X,DONG Y, LUO P,et al. Determination of benzimidazole residues in bovine milk by ultra-high performance liquid chromatography - tandem mass spectrometry[J]. J Chromatogr B, 2010(878): 3174—3180.

[18] CVILINK V,SKÁLOVÁ L,SZOTÁKOVÁ B,et al. LC - MS - MS Identification of Albendazole and Flubendazole Metabolites Formed Ex Vivo by Haemonchus Contortus[J]. Anal Bioanal Chem, 2008(391):337—343.

[19] MARTINEZ V J, GARRIDO F A, AGUILERA-LUIZ M M, et al. Development of Fast Screening Methods for the Analysis of Veterinary Drug Residues in Milk by Liquid Chromatography-Triple Quadrupole Mass Spectrometry[J]. Anal Bioanal Chem, 2010,397(7):2777—2790.

[20] STOLKER A A, PETERS R J, ZUIDERENT R, et al. Fully Automated Screening of Veterinary Drugs in Milk by Turbulent Flow Chromatography and Tandem Mass Spectrometry[J]. Anal Bioanal Chem, 2010,397(7):2841—2849.

[21] Guidance for Industry—Bioanalytical Method Validation (2001) U. S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), Rockville, MD, USA[EB/OL]. 2011-07-31[2012-11-12]. http://www. fda. gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107. pdf.