



Multi-walled carbon nanotubes as solid-phase extraction adsorbent for determination of albendazole and its metabolites in shrimp and crab by ultra-performance liquid chromatography tandem mass spectrometry

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ABSTRACT

An effective and sensitive method was developed for simultaneous determination of albendazole and its three metabolites in shrimp and crab using multi-walled carbon nanotubes (MWCNTs) as solid phase extraction (SPE) sorbent coupled with ultra-performance liquid chromatography-tandem mass spectrometry. In the whole procedure, the multi-isotope dilution method was used to correct the loss during the SPE and variations associated with the analysis. Under optimum conditions, the method was validated with respect to linearity, specificity, accuracy and precision. The linearity of the method was good in the range from 0.1 to 20 ng/mL, with regression coefficients all above 0.9986. The limits of detection and quantification for the four analytes were 0.02-0.04 and 0.06-0.12 ng/mL. The mean recoveries in both matrices spiked at range from 0.2 to 5.0 ng/g were 95.2-104.0%, with intra-day RSDs less than 5.26%, and inter-day RSDs less than 4.25%. The method was later successfully applied to real shrimp and crab samples for the determining and confirming of albendazole and its three metabolites.

Keywords: albendazole, multi-walled carbon nanotubes, solid-phase extraction, UPLC-MS/MS.

INTRODUCTION

Albendazole ([5-(propylthio)-1H-benzimidazol-2-yl]carbamic acid methyl ester) is a potent broad-spectrum benzimidazole anthelmintic agent widely used against intestinal helminth infections in mammals [1]. Following oral dosing to farm animals, albendazole is readily absorbed from the gut and rapidly metabolized by oxidation of its sulfide group to form albendazole sulfoxide, albendazole sulfone and albendazole 2-aminosulfone. Toxicological studies in both farm and laboratory animals have shown ABZ and its active metabolite ABZSO to be teratogenic. In veterinary practice, ABZ is used in mammals but not in aquatic product. While in recent years, ABZ was sometimes used for helminths infections in aquaculture, and the residue may pose health risks to consumers. Maximum residue limit (MRL) of 100 µg/kg for muscle has been set both by the European Union and China.

Over the years, many methods have been described for the analysis of ABZ and its metabolites in biological matrices using HPLC with ultraviolet detection [2-4] and fluorometric detection [5-9]. Nowadays, high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method [10-20] has found more widespread application in ABZ and its metabolites analysis by offering more sensitive detection and increased

confidence in confirmation. Previously, we reported an rapid, specific and sensitive UPLC-MS/MS method to determine ABZ and its metabolites in fish tissue, the UPLC technique offers reduced run time and improved sensitivity[21].

In recent years, multi-walled carbon nanotubes (MWCNTs), a new kind of carbon material, have attracted much interest that is directed toward the development of solid-phase extraction (SPE) adsorbents [22]. MWCNTs potentially used as a suitable SPE sorbent are primarily owned to their ability to establish π - π interactions, excellent Van der Waals interactions with other molecules [23] and particular structure with internal tube cavity[24]. Till now, MWCNTs have been successfully used as the sorbent for the determination of heavy metals[25-27], organic compounds [28-30]. But to our knowledge, the SPE performance of MWCNTs using for the determination of ABZ and its metabolites has not been previously investigated.

In this study, a fast and sensitive method, in which MWCNTs were used as effective adsorbents for SPE coupled with UPLC-MS/MS, was developed for the simultaneous determination of ABZ and its metabolites in shrimp and crab samples.

EXPERIMENTAL SECTION

Chemicals and reagents

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, ABZSO₂, ABZ-2-NH₂SO₂, deuterated albendazole (D₃-ABZ), deuterated albendazole sulfone (D₃-ABZSO) and deuterated albendazole sulfone(D₃-ABZSO₂) was obtained from Adlershof GmbH (Berlin, Germany; purity \geq 98%). De-ionised water was used throughout the study. MWCNTs were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China) and were dried for 3 h at 130 °C to removing the adsorbed water beforehand and kept in desiccator. New solid-phase extraction (SPE) cartridge tube (empty, 3 mL, polypropylene, with 20 μ m polypropylene upper and lower frits) was purchased from Agela Technologies Co. Ltd. (Shanghai, China).

Sample Preparation

The 2.00 \pm 0.02 g thawed and homogenized sample was weighted into a 50-mL polypropylene tube and then spiked with 100 μ L 100 ng/mL internal standard working solution. After the addition of 100 μ L 10 M sodium hydroxide and 10 mL ethyl acetate, the tube was mixed on a vortex mixer for 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 10 mL ethyl acetate was added to the sample tube, mixed and centrifuged as above. The resulting supernatant was combined and evaporated to dryness and re-dissolved in 5 mL ethyl acetate.

MWCNTs SPE clean-up

The MWCNTs-packed cartridge was prepared in the following step: 20 μ m polypropylene lower frit was placed at the bottom of a new cartridge tube (3 mL, polypropylene), 40 mg MWCNTs was packed, then 20 μ m polypropylene upper frit was placed on the top. The MWCNTs packed height was controlled about 0.5 cm. The flow rate of the solutions was maintained 1 mL/min under vacuum conditions.

A prepared MWCNTs-packed SPE cartridge was pre-washed with 3 mL methanol and 3 mL ethyl acetate. The re-dissolved extracts were transferred directly to the SPE cartridge. The cartridge was washed with 3 mL water, after that the cartridge was dried for 2 min under vacuum. Then, the target analytes retained on the MWCNTs were eluted with 10 mL 5% formic acid in methanol. The effluents were collected into a 10mL glass centrifuge tube and condensed to dryness under a gentle flow of nitrogen at 40 °C and re-dissolved with 1.0 mL of the initial mobile phase. Finally, the solution was filtered through a 0.22 μ m PTFE filter and transferred to a glass vial for UPLC-MS/MS.

UPLC-MS/MS ANALYSIS

Measurements were performed using an ACQUITY ultra-performance LC system (Waters, Milford, MA, USA) in combination with a Quattro Premier XETM Micromass[®] triple-quadrupole mass spectrometer (Manchester, UK). Chromatographic separation was achieved with a UPLC column(BEH C₁₈ 2.1 \times 50 mm, 1.7 μ m particle size). The column temperature was maintained at 40 °C. The mobile phase was used at a flow rate of 0.3 mL/min with solvent

A(methanol) and B (aqueous formic acid 0.2% (v/v)), the gradient elution was performed 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 temperature in the autosampler was set at 10°C and the sample volume injected was 10 µL.

The mass spectrometer was operated in electrospray positive mode, and data acquisition was in multiple reaction monitoring mode(MRM). Analyte and internal standard transition ions and associated mass spectrometric parameters were used according to our previously study[21].

RESULTS AND DISCUSSION

OPTIMIZATION OF THE MWCNTs-SPE PROCEDURE

In order to achieve an adequate extraction performance by using carbon nanotubes as sorbent for SPE, the optimization of several parameters was investigated, including the kind of sorbent employed, MWCNTs amount, loading flow rate, and elution volume. Briefly, 40 mg MWCNTs(40-60 nm) make the MWCNTs-SPE cartridge more effective and much lower than conventional SPE sorbent. 10 mL methanol containing 5% formic acid provide the best recovery and shorter time for SPE elution. The optimized MWCNTs-SPE procedure was shown in section “MWCNTs SPE CLEAN-UP”.

Method Vallidation

The specificity of the method was determined by analysis blank shrimp and crab samples to evaluate possible endogenous interferences. Ten blank samples of each specie were extracted and analyzed using the method, and no interference detected at the retention times of the four analytes. Compared to the previous method[24], this method performed additional MWCNT clean-up procedure, and the TIC chromatogram of the spiked samples (Figure-1) shows less endogenous interference and gives more clearer effect.

The linearity of standard calibratin curve was established by the analysis of standard solutions at six concentrations (0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 ng/g). To each sample 100 µL 100 ng/mL internal standard working solution was added. A linear least-squares regression of the analyte/internal standard peak area ratio to the nominal concentration was constructed, with a weighting factor of $1/x^2$. The linear ranges and correlation coefficients of four analytes are shown in Table-1. As can be seen, the linearity in the range of concentration tested was excellent with correlation coefficients over 0.9986.

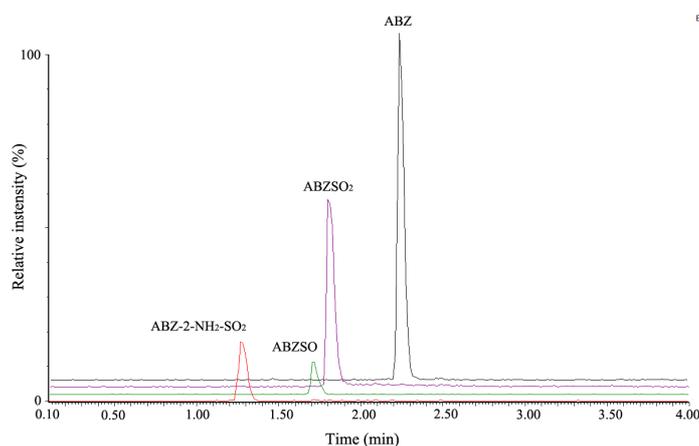


Figure-1: Total ion chromatogram for spiked crab sample at 1.0 ng/g of ABZ, ABZSO, ABZSO₂ and ABZ-2NH₂-SO₂

Table-1: Linear range, correlation coefficient , and limits of detection and quantification

Analyte	Linear regression equation	Correlation coefficient r^2	Liner range (ng/mL)	LOD (ng/g)	LOQ (ng/g)
ABZ	$y=0.3320x+0.0064$	0.9993	0.1-20	0.02	0.06
ABZSO	$y=0.4172x+0.0211$	0.9990	0.1-20	0.02	0.06
ABZSO ₂	$y=0.6716x+0.0364$	0.9986	0.1-20	0.02	0.06
ABZ-2NH ₂ - SO ₂	$y=1.2385x+0.0244$	0.9989	0.1-20	0.04	0.12

Limit of detection(LOD) and limit of quantitation(LOQ) are considered as the analyte minimum concentrations that can be confidently identified and quantified by the method. LOD and LOQ were calculated on the basis of signal to noise ratio $S/N = 3$ for LOD and $S/N = 10$ for LOQ) on the chromatograms of 20 shrimp samples. The method LOD were found to be 0.02-0.04 ng/g and LOQ were 0.06-0.12 ng/g, respectively. These data are listed in Table-1. The concentration of LOQ also meet the FDA guide on Analytical Procedures and Methods Validation, which was defined as the concentration that could be determined with 80-120% accuracy and not higher than 20% precision.

The recovery was determined by experiments using spiked blank matrix with replicates at three concentration levels (0.2, 1.0, 5.0 ng/g). Six determinations were carried out at two investigated tissue(shrimp and crab). Precision were assessed by performing replicate analysis of spiked samples against calibration curves. Mean recovery, intra-day and inter-day precision of the method are reported in Table-2. The result shows good recovery in shrimp and crab tissue, and the recovery of the analytes was not substantially affected by their concentrations. As indicated by the results, the RSD values always remained below 5.26%, and the accuracy of the method did not deviate from 100 % by more than 5%.

METHOD APPLICATION

The method was applied to 40 shrimp and 20 crab samples collected from local markets (Zhoushan, China). The samples were analyzed, and no sample was confirmed positive. Then, this method was used for confirming a positive eel sample we detected last year, a similar result for 0.91 ng/g (ABZ), 0.44 ng/g (ABZSO), and 0.25 ng/g (ABZSO₂) was obtained.

Table-2: Summary of recovery and precision assay results from spiked shrimp and carb samples

species	Analyte	Added (ng/g)	Mean recovery (% , n=6)	Inter-day RSD (% , n=6)	Intra-day RSD (% , n=6)
shrimp	ABZ	0.2	103.2	4.21	3.41
		1.0	104.0	3.70	3.12
		5.0	99.2	3.00	4.13
	ABZSO	0.2	102.8	2.65	2.46
		1.0	101.0	2.44	3.70
		5.0	98.8	2.36	3.24
	ABZSO ₂	0.2	101.8	4.25	2.06
		1.0	99.3	3.78	2.83
		5.0	98.7	1.76	5.26
	ABZ-2NH ₂ - SO ₂	0.2	98.6	3.98	4.08
		1.0	100.7	3.22	3.09
		5.0	99.1	2.04	4.50
crab	ABZ	0.2	100.8	3.99	5.05
		1.0	95.2	3.45	3.59
		5.0	98.3	2.34	2.99
	ABZSO	0.2	100.3	4.15	3.74
		1.0	99.1	3.37	4.26
		5.0	103.0	3.14	4.77
	ABZSO ₂	0.2	99.3	2.78	4.64
		1.0	95.4	3.23	3.82
		5.0	99.2	4.20	4.47
	ABZ-2NH ₂ - SO ₂	0.2	99.2	1.80	2.81
		1.0	100.6	3.48	4.67
		5.0	97.9	2.95	4.14

CONCLUSION

In conclusion, an effective and sensitive method was set up for simultaneous determination of ABZ and its metabolites in shrimp and carb in this work. The MWCNTs was applied as an effective SPE adsorbent for enrichment and clean-up of ABZ and its metabolites. Stable isotope-labeled internal standards was used to correct the matrix effect and variations associated with the analysis. The established method have a great potential to be widely used for the analysis of ABZ and its metabolites at trace level in shrimp, carb and other aquatic products for residue control programs.

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