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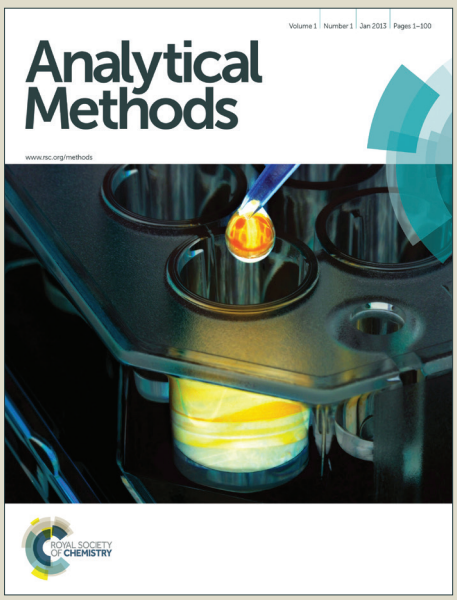

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ARTICLE

Rapid Determination of Tricaine Mesylate Residues in Fish Samples Using Modified QuEChERS and High Performance Liquid Chromatography–Tandem Mass Spectrometry

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A rapid and effective modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method was developed for determination of tricaine mesylate (MS-222) in fish samples using high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). Samples were extracted with the mixture of acetonitrile and acetate buffer, and then cleaned with primary/secondary amino (PSA) absorbents. The determination of MS-222 was achieved in less than 4.0 min using an electrospray ionization source in the positive mode (ESI+). The QuEChERS method was validated by evaluating the repeatability, linearity, precision, trueness. The limit of detection (LOD) was 2.5 μg kg^{-1} and the limit of quantification (LOQ) was 10.0 μg kg^{-1}. The calibration curve was good linear in the range of 2-1000 μg L^{-1} (R^2>0.9999). Average recoveries of MS-222 were in the range of 79.6-119.7%, with a relative standard deviation (RSD) lower than 6%.

1 Introduction

In recent decades, with the development of high-density aquaculture, an increasing number of drugs have been used in the fishery [1]. Although the use of drugs leads to a marked increased production of fishery, it has caused serious drug residues in vivo. Nowadays, the drug residues in aquatic products have been obtaining more and more attentions. Thus, rapid determination of different drug residues in aquatic products is required.

To achieve this aim, various analysis methods have been developed. The sample pretreatment is an important section of an analysis method, which can make the extraction liquid of aquatic product samples clean [2-4]. Up to now, numerous pretreatment methods have been described about samples cleaning of aquatic product such as solid-phase extraction (SPE) [5, 6], dispersive solid-phase extraction (DSPE) [7], soxhlet extraction [8], accelerated solvent extraction (ASE) [9]. However, these pretreatment methods usually involve large volumes of organic solvents to extract drugs and the processes usually take several hours.

In recent years, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method has became increasingly popular due to its simplicity and high throughput compared to other

methods. QuEChERS was developed by Anastassiades in 2003 firstly, which combined acetonitrile partitioning and DSPE technique [10]. Since the QuEChERS method proposed, it has been applied in various fields. Li et al. [7] validated it by analyzing cyflumetofen and its main metabolite residues in plant and pork live using liquid chromatography–mass spectrometry (LC–MS). Lehotay et al. analyzed 229 pesticides successfully in fruits and vegetables using QuEChERS method [11]. Recently, QuEChERS method has been used in fishery field and obtained good results. Rafidah et al. combined the primary/secondary amino (PSA) and octadecyl (C18) as cleanup sorbents to determine avermectins in fish with LC–MS [12]. PSA was also used as cleanup sorbents alone to determine pyrethroid pesticides in fish with gas chromatography [13].

Tricaine mesylate (MS-222) is one of most commonly anesthetic used in fish during blood sampling, artificial propagation, breeding [14]. In America, MS-222 is the only anesthetic which is licensed for use in fish by Food and Drug Administration (FDA) [15]. However, MS-222 is still not licensed in many countries for fishery use such as China. Although MS-222 is not especially toxic to human, a 21-day withdrawal period with fish is still regulated by FDA [15]. The anesthetic effect may occur when people eat too much fish containing high MS-222 residues. Thus, an effective method needs to be developed for the regulation of MS-222. In order to determinate the MS-222 residues in fish, various pretreatment methods have been developed. Scherpenisse Peter et al. developed the determination method of MS-222 residues in fish with solid phase extraction (SPE) using liquid chromatography–tandem mass spectrometry (LC–MS/MS) [16]. Unfortunately, the method was too complicated to be used for the rapid determination of MS-222 in fish samples.

By insight into these works, an interesting trace was found that PSA was a good cleanup sorbents for fish samples using QuEChERS. The PSA could absorb the acidity interferents due to the primary secondary amine on the surface when it was mixed with the extraction liquid of fish samples. With this sense, we started to work on the determination of MS-222 in fish samples using QuEChERS. The extraction solution and amounts of PSA were investigated systematically, with data worth of publication to benefit the researchers in fishery field.

In this study, a modified QuEChERS method was developed for the determination of MS-222 in fish with high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). The fish samples contain complex matrix components such as high protein content and high acidic content. The presented QuEChERS method greatly simplified the sample pretreatment procedure, and the results were satisfactory. The proposed QuEChERS-HPLC-MS/MS method was validated for rapid analysis of MS-222 residues in fish after short-term exposure to MS-222 solution. The development of fisheries applicable QuEChERS method can provide technical support for government regulation.

2 Experimental

2.1 Reagents and apparatus

The analytical standard MS-222 (purity >97%) was obtained from TCI (Tokyo, Japan). Liquid chromatography grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Glacial acetic acid was purchased from Beijing Chemical Reagent Company (Beijing, China). Sodium acetate trihydrate (purity 99-100.5%) was purchased from Alfa. Sodium chloride (NaCl) and anhydrous magnesium sulfate (anhydrous MgSO4) were analytical grade and purchased from Beijing Chemical Company (Beijing, China). Primary/secondary amino (PSA) absorbents (40-63 μm, 60 Å) were purchased from Anpel Scientific Instrument Co. Ltd (Shanghai, China). Ultra-pure water was obtained from a Milli-Q system (Millipore Corporation, MA, USA). Standard stock solutions of MS-222 (100 mg kg^{-1}) were prepared in pure methanol. Standard working solutions at 1-1000 μg kg^{-1} concentrations were prepared from the stock solution by serial dilution. All solutions were stored in a refrigerator at -20 ◦C until use.

A homogenizer (IKA, Germany), a centrifuge (Sigma, America), and a vortex mixer (IKA, Germany) were used during sample preparation. Extracts were filtered using a polytetrafluoroethylene syringe filter with a pore size of 0.22 μm, purchased from Anpel Scientific Instrument Co. Ltd (Shanghai, China).

2.2 Sample Preparation

Carp samples were obtained from a local market (Beijing, China). Muscle, liver tissue and blood were taken for analysis. They were first homogenized in a homogenizer and stored at −20 ˚C until analysis. The weighed homogenized sample (2.0 g) was placed into a 50 mL centrifuge tube. 100 μL of a standard solution was added to the blank sample for recovery studies to validate the method. The samples were vortexed for 1 min, then 10 mL extracting solution was added followed by vortexing for 1 min. The extraction solution was obtained by mixing 30% (V/V) acetate buffer (pH 4.0) and 70% (V/V) acetrontrile. Subsequently, 1.0 g of NaCl and 4.0 g of anhydrous MgSO4 were added to salt out the proteins of the samples and eliminate water. The tubes were capped and immediately vortexed intensively for 1 min and then centrifuged at 10000 rpm for 10 min. Next, 1.0 mL of the supernatant was transferred into a 2.0 mL centrifuge tube containing 300 mg of PSA sorbent. The samples were again vortexed for 1 min followed by centrifugation at 10000 rpm for 5 min. The resulting supernatant was filtered through a 0.22 μm polytetrafluoroethylene syringe filter into an auto sampler vial for HPLC–MS/MS injection. The flow diagram for QuEChERS pretreatment was shown in Fig. 1.

Real samples preparation was done by short-term exposure to MS-222 solution. The living carp, eel and turbot samples were purchased from a local market (Beijing, China). Fish samples were transported to the laboratory and introduced into a tank containing 5 L MS-222 solution at 400 μg L^{-1} (room temperature). MS-222 solution was obtained by dissolving MS-222 in water. The solution was supplied with compressed air through an air pump. The levels of MS-222 in Muscle, liver and blood were investigated after one hour.

2.3 HPLC-MS/MS analysis

Chromatographic separation was carried out on a Thermo Accla HPLC binary solvent pumps equipped with Kromasil HPLC C18 column (2.1 mm × 50 mm, 3μm particle size). The mobile phase was composed of 5‰ formic acid solution (phase A), methanol (phase B) and acetonitrile (phase C) at a flow rate of 0.3 mL/min and 5.0 μL of sample was injected in each case. The gradient program was as follows: 0-2 min, phase B was 2%(V/V) and phase C was 8%(V/V); 2-2.2 min, phase B was from 2%(V/V)to 16%(V/V) and phase C was from 8%(V/V) to 64%(V/V); 16%(V/V) phase B and 64%(V/V) phase C were maintained for 2.8 min; then phase B was returned to 2%(V/V) and phase C was returned to 8%(V/V) in 5.2 min. Finally, 2% (V/V) phase B and 8%(V/V) phase C were maintained for 0.8 min for reconditioning the column prior to the next injection. The column oven temperature was maintained at 40 ˚C to decrease viscosity.

A triple quadrupole mass spectrometer (Thermo Corp., Milford, MA, USA) equipped with an ESI source was used to analyze MS-222 in the positive selected-ion monitoring mode. The MS monitoring conditions were typically as follows: the capillary voltage 3.2 kV; sheath gas pressure 45 Arb; auxiliary gas pressure 10 Arb; the capillary temperature and vaporizer temperature were held at 350 ˚C and 200 ˚C, respectively. Selected reaction monitoring (SRM) was used for the detection of all compounds. According to the European Union Commission Decision 2002/657/EC, conformation for the identification of MS-222 requires at least 1 precursor ion and 2 product ions [17]. Also, identification was made based on the retention time. The precursor ions, product ions, corresponding collision voltages, and tube lens were listed in Table 1. The SRM diagram of standard MS-222 solution was shown in Fig. 2.

Table 1 Mass spectrometry parameters for quantization and confirmation[c]

Analyte	Retention time (min)	Q1 mass (m/z)	Q3 mass (m/z)	SRM Collision energy (v)	Tube lens(v)
MS-222	3.77	166	138*	16	78
			77	26	78

[c] The ion marked with asterisk was for quantification

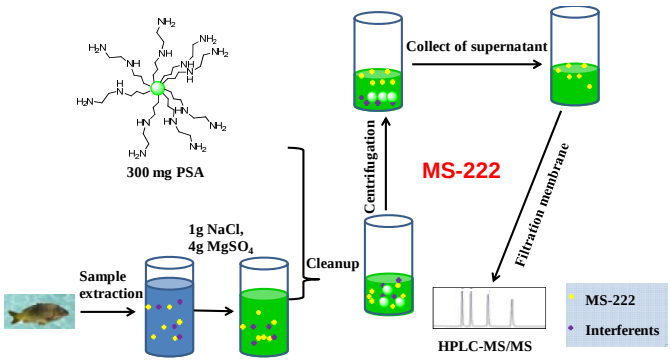

Fig. 1 Flow diagram for QuEChERS pretreatment.

3 Results and discussion

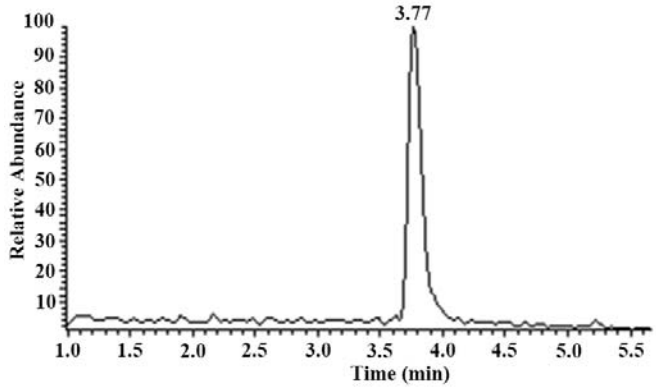

Fig. 2 SRM chromatogram of standard MS-222 solution at 10 μg L^{-1}.

3.1 Optimization of Extraction Solvents

MS-222 is soluble in organic solvents (i.e., methanol, acetonitrile, and ethyl acetate). In the preliminary experiment, different organic solvents were evaluated. In order to promote salting out the proteins, different volumes of acetate buffer solution was added to acetonitrile used as the extraction solution. The volume fraction of acetate buffer was evaluated using spiked fish muscle of 50 μg kg^{-1} (Fig. 3). The results showed that the average recovery rate of MS-222 was up to 132.7% when using pure acetonitrile as extraction solution compared with the mixture of acetonitrile and acetate buffer (pH 4.0), which were 91.0-112.1%. The volume fraction of acetate buffer in acetonitrile was related to the recoveries of MS-222. To achieve best extraction results, 30% of acetate buffer solution was selected to compose the extraction solution.

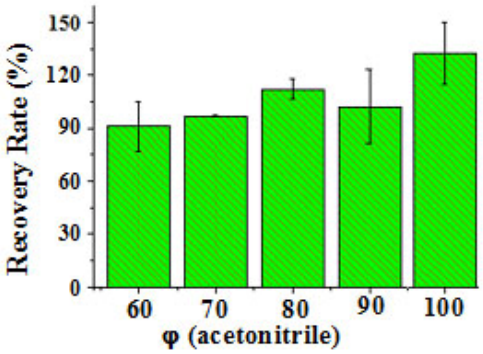

Fig. 3 Effect of volume fraction (ψ) of acetonitrile on the recovery of MS-222 using QuEChERS and HPLC–MS/MS from spiked samples at 50μg kg^{-1}.

3.2 Optimization of the Amounts of PSA

The surface of PSA contains lots of primary/secondary amino groups which could selectively adsorb fatty acids. PSA has been used to clean tobacco samples [18]. When it was mixed with the extracted solution of fish samples, PSA could absorb and remove the interferents such as fatty acids. In this study, we evaluated the cleaning effect of PSA by comparing the recovery of spiked fish muscle of 25 μg kg^{-1}. During the optimization of sample purification, PSA with different amounts were evaluated. The results from experiments conducted with five different amounts of PSA (i.e., 100, 200, 300, 350 and 400 mg) were compared. As shown in Fig. 4, the 300 mg of PSA displayed best clean-up performance. When the amount of PSA was 300 mg, the recoveries of MS-222 were kept in the acceptable range. When the amount of PSA was lower than 300 mg, the instrument signal of MS-222 could be suppressed by matrix effect and led to the low recovery rate. When the amount of PSA was higher than 300 mg, PSA was so much that MS-222 could be absorbed and led to the low recovery rate. The results showed that the optimal purification of the matrices is achieved with 300 mg of PSA. Consequently, 300 mg was used as the optimum amount of PSA in further studies because acceptable recoveries were obtained at this amount.

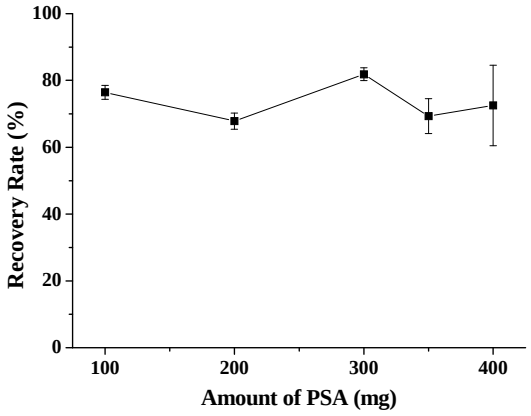

Fig. 4 Effect of the amount of PSA on the muscle clean-up properties (n=3).

3.3 Method performance

The following parameters of the method were evaluated: sensitivity, linearity, and recovery.

The sensitivity was evaluated by determining the limit of detection (LOD) and the limit of quantification (LOQ). The linearity was determined by injecting six standards solution directly into HPLC-MS/MS in triplicate, ranging from 1 to 1000 μg L^{-1}. External calibration curve was achieved in the range of 2 to 1000 μg L^{-1}, and the value of correlation coefficient (R^2) of the linear regression was higher than 0.9999. The LOD and LOQ were calculated based on the signal-to-noise ratio (S/N) of 3.0 and 10, respectively [17]. The LOD and LOQ were 2.5 μg kg^{-1} and 10.0 μg kg^{-1}, respectively (Table 2).

To evaluate matrix effect on the established method, three different concentrations (25, 50 and 500.0 μg kg^{-1}) of MS-222 were spiked into the blank samples. The recovery assays were conducted to investigate the trueness and precision of the method. The blank samples were verified to be free of MS-222 by HPLC-MS/MS. Different fish tissues (muscle, liver, blood) were studied with the same QuEChERS pretreatment method. Three replicates

Table 2 Tissues tested by HPLC-MS/MS with PSA clean-up included muscle, blood and liver (n=3)[d]

Tissues	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Recoveries (%)		
			25 μg kg^{-1}	50 μg kg^{-1}	500 μg kg^{-1}
muscle	2.5	10.0	119.7 (2.6)	97.0 (0.5)	94.5 (1.6)
liver	2.5	10.0	86.2 (5.3)	87.8 (5.1)	85.6 (3.2)
blood	2.5	10.0	98.2(3.2)	85.6 (3.3)	79.6 (1.5)

[d] Bracket is RSD in %.

of the spiked fish tissues at these three different levels were prepared. The precision under these conditions of repeatability, expressed as the RSD, was determined (Table 2). All recoveries were satisfactory, with mean values ranging from 70% to 120%, and the relative standard deviation (RSD) below 6%. The results suggested that the developed QuEChERS method with HPLC-MS/MS was reliable and sensitive to simultaneously quantify MS-222 in fish samples.

3.4 Application to Real Samples

The newly developed QuEChERS method was applied to analyze real samples. The fish samples were firstly introduced into MS-222 solution at 400 μg L^{-1} for one hour, and then the levels of MS-222 were detected (Table 3). MS-222 was detected in all tissues but liver of carp and liver and blood of eel presented concentrations lower than LOQ.

Table 3 Concentration (μg kg^{-1}) of MS-222 detected in fish samples after short-term exposure to MS-222 solution

Real samples	Tissues		
	Muscle	Blood	Liver
Carp	21.7	150.1	-[e]
Eel	26.7	-[e]	-[e]
Turbot	38.1	85.1	185.3

[e] Lower than LOQ.

4 Conclusions

The work represents the first application of QuEChERS for the extraction of MS-222 residue in fish tissues. Extracts were analyzed and validated using HPLC–MS/MS in the ESI positive mode. This method provided analysis of MS-222 within 4.0 min with good specificity. The recovery percentages were between 79.6 and 119.7% (RSD <6%) in fish tissues, and the LOQ were in 10 μg kg^{-1}. The results show that the PSA was the appropriate sorbent obtaining the good analysis of particularly complex matrices. The satisfactory result was obtained from the analysis of real samples. The QuEChERS method was an effective method to determine MS-222 in fish and could be used for MS-222 residues monitoring in fish origin samples.

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Notes and references

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