Original Article

Determination of sulfamonomethoxine in tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) by liquid chromatography-tandem mass spectrometry and its application pharmacokinetics study

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ABSTRACT

A precise and reliable analytical method to measure trace levels of sulfamonomethoxine (SMM) and N^4-acetyl metabolite in tilapia samples using liquid chromatography-tandem mass spectrometry was developed. Optimized chromatographic separation was performed on C18 reversed-phase columns using gradient elution with methanol and 5 mmol/L of an ammonium acetate aqueous solution (adjusted to pH 3.5 using formic acid). This study investigated the pharmacokinetic properties and tissue distribution of SMM and its major metabolite N^4-acetyl sulfamonomethoxine (AC-SMM) in tilapia after a single dose of 100 mg kg^{-1} body weight of orally administered SMM. Blood and tissues were collected between 0.5 and 192 h with 14 total sampling time points. SMM was rapidly absorbed, and extensively distributed in the bile and liver through systemic circulation. Enterohepatic circulation of SMM was observed in the tilapia body. Acetylation percentages were 45% (blood), 90% (liver), 62% (kidney), 98% (bile), and 52% (muscle). High concentrations of AC-SMM accumulated in the tilapia bile. At 192 h, AC-SMM concentration in the bile remained at 4710 µg kg^{-1}. The k_e value of AC-SMM (0.015 h^{-1}) in the blood was lower than that of SMM (0.032 h^{-1}). This study demonstrated effective residue monitoring and determined the pharmacokinetic properties of SMM and AC-SMM in tilapia.

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1.   Introduction

Sulfonamides (SAs) are a group of synthetic antibacterial agents that are effective against bacterial infections. These drugs can improve the productivity of cultured organisms because of their cost-effectiveness and wide-spectrum antimicrobial activity [1]. Done and Halden [2] indicated that SAs are often used for the treatment of fish in the United States. In addition, SAs are one of several vital antibacterial agents used to prevent and treat aquaculture diseases in Taiwan [3]. SA residues may occur in animal tissues if the required withdrawal times are not followed or if the SAs are improperly administered [4]. Therefore, the presence of SAs in the fish supply has attracted considerable attention. Continual exposure to these compounds may result in the accumulation of the parent compound, their metabolites, or both, in the tissue of marine products. Consuming contaminated animal products can cause allergic reactions in people and negatively affect the human immune system [5]. The discovery of drug residues of animal origin in food has compelled the United States, the European Union, Japan, and many countries worldwide to establish monitoring programs and maximum residue limits (MRLs). For example, the European Union has set an MRL of 0.1 mg kg^{-1} for SAs in animal food products [6], and Taiwan has set an MRL of 0.1 mg kg^{-1} for sulfamonomethoxine (SMM) and sulfadimethoxine in aquatic food products [7].

In pharmacokinetics (PK) of SA studies, substantially more information is available regarding N^4-acetylation than N^4-hydroxylation because acetylation is the primary elimination pathway in many species and synthesis of the hydroxyl group is difficult [8]. The properties of acetylated SAs are as follows: (1) They have lower water solubility than that of the parent compound and may lead to renal toxicity due to their precipitation in the kidneys [9,10]; (2) they exhibit no antibacterial activity [9,11]; (3) they can be deacetylated in organisms [8,9]; and (4) plasma protein binding is higher than that in the parent compound, which may affect their excretion rates [10,11].

Regarding SAs, numerous PK profile studies have been reported in various aquatic animal species, namely shrimp (*Fenneropenaeus chinensis*) [12], summer flounder (*Paralichthys dentatus*) [13], walleye (*Sander vitreus*) [13], eel (*Anguilla anguilla*) [14], rainbow trout (*Oncorhynchus mykiss*) [15], yellowtail (*Seriola quinqueradiata*) [15], Atlantic salmon (*Sdmo sahr*) [16], and penaeid shrimp (*Penaeus vannamei*) [17].

Many studies have investigated SA analytical methods in biological samples [18–20]. However, few have explored SA metabolites, such as N^4-acetyl SMM, which can lead to renal toxicity through precipitation [9,10]. Therefore, in this study, SMM and N^4-acetyl sulfamonomethoxine (AC-SMM) were determined using liquid chromatography-tandem mass spectrometry (LC–MS/MS).

Tilapia is one of the most economically crucial farmed fish species and a major aquatic export of Taiwan [21]. SMM is the most common sulfonamide drug used in aquaculture; however, few studies have characterized the PK disposition of SMM in tilapia, particularly AC-SMM. Understanding the absorption, distribution, metabolism, and excretion of SMM and its N^4-acetyl metabolite is essential in drug residue and food safety. Thus, the objective of the present study was to evaluate the PK profile of SMM and AC-SMM in tilapia after administration of SMM in a single oral dose of 100 mg kg^{-1} body weight.

2.   Materials and methods

2.1.   Chemicals, reagents, and solutions

SMM was purchased from Sigma–Aldrich (Steinheim, Switzerland). AC-SMM was synthesized in the laboratory using SMM as described in a relevant study [22], and characterized using proton nuclear magnetic resonance (Fig. S1) and electrospray ionization mass spectrometry (Fig. S2). Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Formic acid (99%) and ammonium acetate were obtained from Fluka (Berlin, Germany). C18 powder was obtained from J.T. Baker (Phillipsburg, NJ, USA). Water was deionized using a Millipore purification system (Millipore, Billerica, MA, USA). Standard SMM and AC-SMM stock solutions (100 μg mL^{-1}) were prepared in methanol and stored at −20 °C. A working standards solution was prepared by diluting the standard stock solution in methanol to a concentration of 0.1 μg mL^{-1}.

2.2.   LC–MS/MS conditions

The LC–MS/MS conditions and extraction method were modified from a relevant study [23]. The LC–MS/MS system comprised an Agilent Series 1100 LC system (Agilent Technologies, Waldborm, Germany) connected to a Sciex API 4000 QqQ mass spectrometer (Applied Biosystems, Foster City, CA, USA). The parameters applied to the mass spectrometer in the positive ion mode were as follows: an ion source block temperature of 650 °C, an electrospray capillary voltage of 5500 V, and a collision gas pressure of 5 mTorr using nitrogen as the gas. The most intensive ionic fragment from a precursor ion was used for quantification, followed by a less sensitive secondary transition used for confirmation. All data were acquired using Analyst Software (Version 1.4, Applied Biosystems/MDS Sciex).

LC analysis was performed on a C18 reverse-phase column (4.6 × 150 mm, 5 μm; Agilent, ZORBAX SB-C18) at room temperature. The column was used at a constant flow rate of 0.5 mL min^{-1}. The mobile phase comprised a 5 mmol/L solution of ammonium acetate and formic acid with an adjusted pH of 3.5 (Solvent A) and methanol (Solvent B). Linear gradients of 5%–60% B (0–5 min), 60% B (5–7 min), 60%–80% B (7–10 min), 80% B (maintenance, 10–12 min), and 80%–5% B (12–15 min) were used, and the injection volume was 20 μL.

2.3.   Sample extraction procedures

Tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) samples were obtained from a commercial farm. The collected biological samples were preliminarily confirmed to be free from contamination of SA residues by LC–MS/MS analysis after sample handling with the proposed methods described

in the sections that follow. The experimental methods were conducted by spiking the fish samples with a mixture of SMM and AC-SMM at a concentration of 100 μg kg^{-1}. All of the fish samples were homogenized and stored at −20 °C until analysis was conducted.

Blood (200 μL), liver (0.2 g), and bile (0.2 g) samples were placed in 2-mL centrifuge microtubes and mixed with 1 mL of acetonitrile; the samples were shaken vigorously and then vortexed for 5 min at room temperature. After extraction, the samples were centrifuged (4500 × g) for 10 min. Subsequently, the supernatant was transferred to a new centrifuge tube, C18 sorbent (20 mg) was added, and the mixture was homogenized for 30 s and centrifuged (4500 × g) for 5 min. The supernatant was evaporated to dryness under nitrogen gas at 37 °C, and the samples were reconstituted in 1 mL of acetonitrile. Finally, the samples were filtered through a 0.22-μm filter membrane (Millipore, USA), and 20 μL of the aliquot was injected into the LC−MS/MS.

Homogenized fish muscle samples (1 g) were accurately weighed into a 50-mL polypropylene centrifuge tube, and 5 mL of acetonitrile was added. The samples were shaken vigorously and then vortexed for 15 min at room temperature. After extraction, the samples were centrifuged (4500 × g) for 10 min. Subsequently, 1 mL of the supernatant was placed in a new centrifuge tube; C18 sorbent (20 mg) was added, and the mixture was homogenized for 30 s and centrifuged (4500 × g) for 5 min. The supernatant was filtered through a 0.22-μm filter membrane (Millipore, USA), and 20 μL of the aliquot was injected into the LC−MS/MS system.

2.4. Analytical performance

The analytical method was validated [23] using the tilapia samples as the primary matrix. The following parameters were assessed for SMM and AC-SMM: linearity, recovery, precision, and specificity. Analytical curves were constructed to estimate linear ranges, correlation coefficients, and detection and quantification limits for the proposed method. The linearity of the method was evaluated by calculating the regression line and was expressed using the correlation coefficient (R) (n = 3). Recovery experiments were then performed by comparing the analytical results of the extracted tilapia samples. SMM and AC-SMM were added at levels of 100 μg kg^{-1} before the extraction procedure, with the standard solutions prepared at the same concentration without real samples, representing 100% recovery (n = 3). The precision (repeatability) of the method was determined by repeatedly analyzing fish samples spiked at concentrations of 100 μg kg^{-1} and then calculating the relative standard deviation (RSD, %) of the measurements (n = 3). Finally, specificity was determined through the analysis of SMM and AC-SMM in 20 blank fish tissue samples and evaluation of potential endogenous interferences.

2.5. Pharmacokinetics studies

2.5.1. Drug administration and sampling
Healthy tilapia (*O. niloticus* × *O. mossambicus*) weighing approximately 500 ± 50 g were acclimated in a 1000-L tank with a flow of aerated brackish water at a salinity of

approximately 8‰. Water temperature was maintained at 25 °C, and oxygen levels were saturated using an inflation pump. During the PK studies, the fish were gavaged with an aqueous solution (3 mL) of 100 mg SMM/kg body weight. Three fish were sampled (n = 3) at each time point, and samples were collected at 0.5, 0.75, 1, 4, 8, 15, 24, 48, 72, 96, 120, 144, 168, and 192 h after oral administration.

2.5.2. Pharmacokinetics analysis
The PK parameters of the analytes were determined using an noncompartmental analysis model based on statistical moment theory [24–26]. The output of such models includes major kinetic parameters, such as peak plasma concentrations (C_{max}), time to reach peak concentration (T_{max}), area under the plasma concentration−time curve (AUC), terminal elimination half-life ($T_{1/2}$), and terminal rate constant (k_e). The C_{max} and T_{max} of the drug were determined using individual plasma concentration−time curves. Subsequently, k_e was estimated through the linear regression of logarithmically transformed terminal data points. When enterohepatic circulation was believed to occur, k_e was determined using linear regression analysis of a portion of the points in the elimination phase. The terminal points in SMM were from 24 to 96 h in the blood, liver, and kidneys; from 24 to 192 h in bile; and from 24 to 120 h in muscle tissue. The terminal points in AC-SMM were from 72 to 192 h in the blood; from 24 to 144 h in the liver; from 24 to 120 h in the kidneys; from 48 to 192 h in bile; and from 24 to 72 h in muscle tissue. The equations for the AUC and $T_{1/2}$ were as follows:

$$AUC = \int\limits_{0}^{t} Cpdt \tag{1}$$

$$T1/2 = \frac{0.693}{ke} \tag{2}$$

2.5.3. Calculation of acetylation in blood and tissues
The AUC represents the total drug exposure over time and is useful for determining the average concentration over a time interval. Acetylation (%) is calculated by dividing the total AUC (i.e., $AUC_{AC−SMM}$) by AUC_{SMM} + $AUC_{AC−SMM}$ and then multiplying the result by 100%. The equation is as follows [14]:

$$Acetylation(\%) = \frac{AUC_{AC−SMM}}{AUC_{SMM} + AUC_{AC−SMM}} 100\% \tag{3}$$

3. Results and discussion

3.1. Optimization of LC−MS/MS method and analytical performance

Ionization mode (positive or negative ions) and a precursor ion were selected according to the chemical ionization characteristics of the drugs, and parameters of the mass spectrometer were optimized through a direct continual pump infusion of the standard working solutions of the analytes (100 μg kg^{-1}) individually at a flow rate of 10 μL min^{-1} in the mass spectrometer. The results revealed that SMM and AC-SMM exhibited a considerably higher abundance quantity of

$[M+H]^+$ compared with $[M-H]^-$; $[M+Na]^+$ or $[M + NH_4]^+$ was also detected, but with a lower intensity. MS/MS with a multiple reaction monitoring (MRM) mode was the first choice because it accurately, sensitively, and simultaneously quantifies targeted molecules from complex samples. The MS/MS fragmentation of SMM and AC-SMM was investigated by recording the full-scan product ion spectrum of each analyte as a function of the collision energy. Fig. S3 depicts the selected protonated molecular ion $[M+H]^+$ and the potential product ions of SMM and AC-SMM. Typical SA MS/MS fragmentation patterns, including m/z 156, 108, and 92, were observed; other MS/MS fragmentation patterns, including m/z 198, 134, and 108, represented N^4-acetyl metabolites. Fig. S4 displays a typical MS/MS fragmentation ion signal of the protonated SAs and the possible structures of each product ion. Table S1 lists the results of the precursor ion, product ion, and MS/MS parameters, which were selected when optimizing the MRM detection of the analytes.

Selecting the appropriate solvent system is crucial for obtaining optimal separation of components. Various chromatographic conditions were tested to separate the target compounds and obtain a favorable peak shape, high resolution, and high sensitivity in the LC system. The pH value and salt concentration were the major factors; therefore, modification of the aqueous mobile phase for the analytes was evaluated by applying pH values of 3.5 and 7.0 using formic acid and two concentrations of ammonium acetate (5 and 50 mmol/L).

The results revealed that the retention times of SMM and AC-SMM at pH 3.5 were 10.68 and 11.39 min, respectively, and those at pH 7.0 were 10.16 and 10.14 min, respectively (Fig. 1). The reaction at the pH of 3.5 had a higher intensity than that of 7.0, which indicated that the baseline separation was effective. This was because SAs are polar molecules with amphoteric properties; moreover, pK_{a1} (with values of 2–3) and pK_{a2} (with values of 5–11) correspond to the protonation of the aniline group and deprotonation of the sulfonyl amide group, respectively [27]. According to the pK_a value of SMM (Fig. S5) [27], most SAs were positively charged at pH less than 2.5, negatively charged at pH higher than 6.0, and neutral between pH 2.5 and 6.0. Mobile phase A was adjusted to pH 3.5 (analytes in neutral form); therefore, the analytes were likely to interact with the stationary phase in reverse-phase chromatography and achieve superior separation. By contrast, under alkaline conditions (pH > 7.0), the analytes were negatively charged ions, which increased polar properties. They were easily eluted in the C18 reversed-phase column, and thus, did not separate in the LC system. Notably, AC-SMM was less polar than SMM because of the acetylation of the 4-amino group, and the retention time of AC-SMM was slightly longer than that of SMM. In the salt concentration tests alone, mobile phase A consisted of 5 mM ammonium acetate with a satisfactory ionization efficiency; however, a higher salt content indicated a considerable loss of MS sensitivity in SMM during measurement (Fig. 2). Therefore, mobile phase A consisted of a 5 mmol/L ammonium acetate and formic acid solution, with an adjusted pH of 3.5. Conversely, according to a review study [28], most mobile phase Bs consist of acetonitrile or methanol. In this study, methanol was selected for mobile phase B. A chromatogram of the mixtures is shown in Fig. S6.

In the quality assurance and quality control, the analytical performance results exhibited excellent linear relationships (Fig. S7) and favorable correlation coefficients (R = 0.9995 (SMM) and R = 0.9993 (AC-SMM)). The recovery of SMM in various tissues ranged from 77.0% to 85.0%, and RSD values were lower than 10%. Conversely, the recoveries of AC-SMM in various tissues ranged from 88.7% to 97.3%, and RSD values were lower than 11.5% (Table S2). Furthermore, the specificity of the method was tested by analyzing 20 blank samples. The
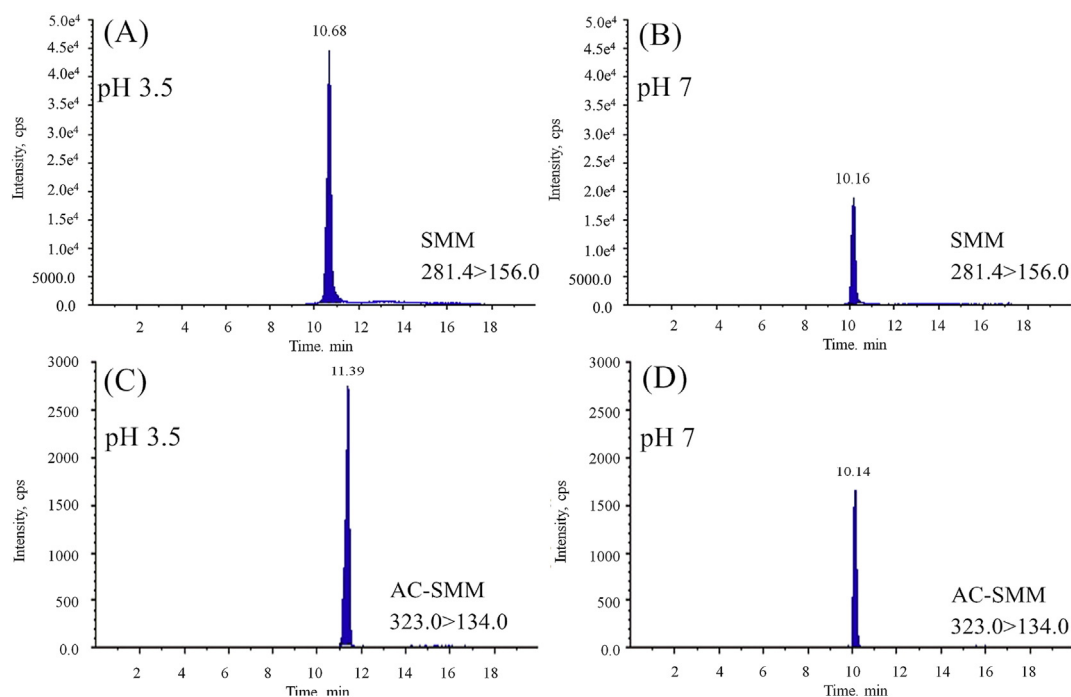
Fig. 1 – Signal intensity of SMM and AC-SMM under various pH conditions: SMM, pH 3.5 (A); SMM, pH 7 (B); AC-SMM, pH 3.5 (C) and AC-SMM, pH 7 (D).
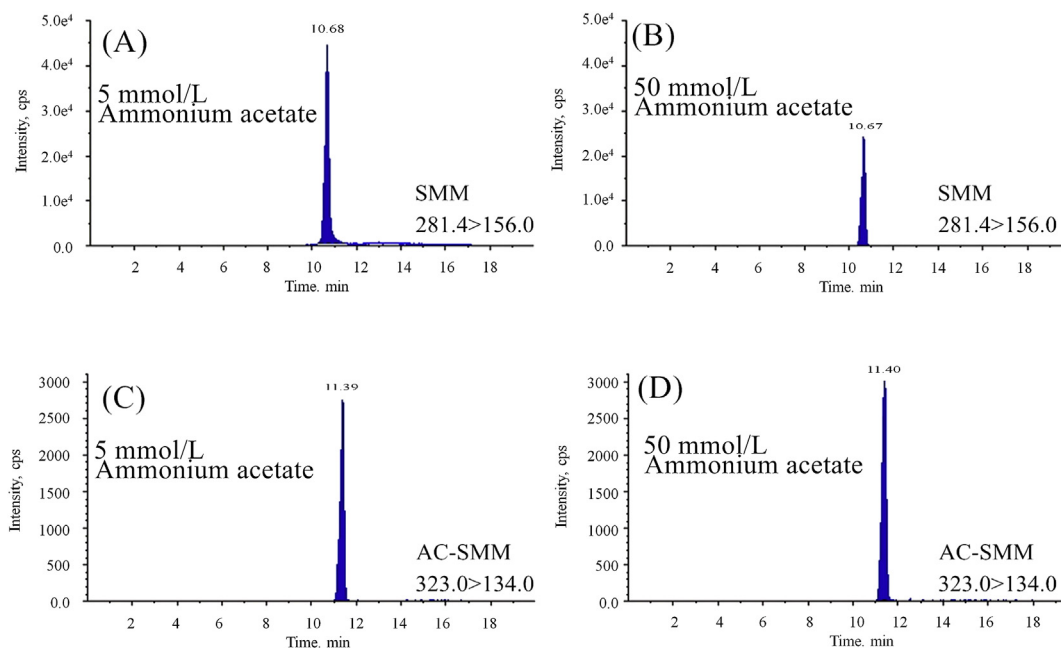
Fig. 2 — Signal intensity of SMM and AC-SMM at various ammonium acetate concentrations: SMM, 5 mmol/L (A); SMM, 50 mmol/L (B); AC-SMM, 5 mmol/L (C) and AC-SMM, 50 mmol/L (D).

simultaneous absence of chromatographic peaks for the fish sample extracts, and retention times for the target analytes, signified that matrix compounds that might produce a false-positive signal were absent in these blank samples.

3.2. Absorption, distribution, and elimination of SMM

The mean concentration of SMM versus time in the blood and tissues is shown in Fig. 3 and the PK parameters of SMM are shown in Table 1. The SMM blood concentrations increased rapidly with time, from 0.5 to 1 h. C_{max} was reached at 1 h, and then the concentration decreased from 24 to 96 h. The redistribution phase of SMM was observed from 96 to 120 h. In the
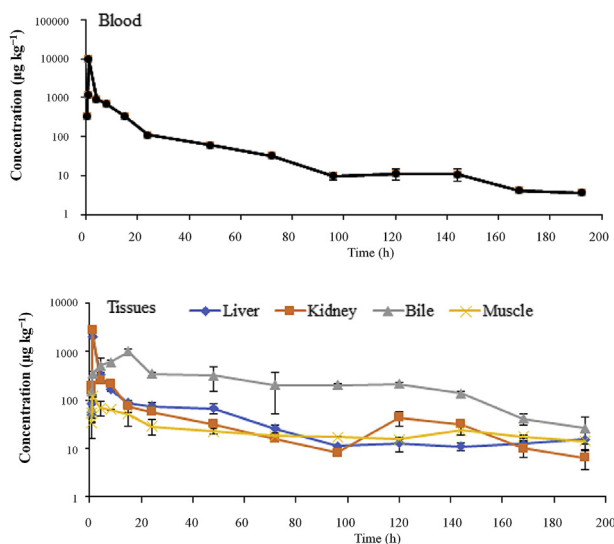
Fig. 3 — Semilogarithmic concentration—time curves of SMM in blood and tissue.

liver, C_{max} was reached at 1 h. Minor increases in SMM concentrations were again observed at the final sampling time (192 h), which were likely caused by deacetylation enzymes in the liver [8,9]. In the kidney, C_{max} was reached at 1 h, and SMM concentration increased from 96 to 120 h and then decreased. In the bile, C_{max} was reached at 15 h and SMM concentration decreased slowly during the elimination phase. At 192 h, SMM concentration in the bile remained at 26.7 µg kg^{-1}. In the muscle, C_{max} was reached at 1 h and SMM concentration was depleted during the elimination phase. Following oral administration, SMM was rapidly absorbed in the tilapia and T_{max} was 1 h (except in the bile).

C_{max} indicates that the systemic absorption of a drug provides a therapeutic response. The C_{max} of SMM in order of magnitude was blood > kidney > liver > bile > muscle. The minimum inhibitory concentrations (MICs) of SMM for pathogenic bacteria such as *Aeromonas salmonicida*, *Aeromonas hydrophila*, and *Vibrio anguillarum* are between 1.6 and 3.2 mg L^{-1} [14]. The C_{max} in the blood (9.57 mg L^{-1}) was above the MICs. It was practical to obtain therapeutic blood concentrations of SMM in tilapia using oral administration of SMM 100 mg kg^{-1} body weight. This may be effective in therapy for tilapia diseases. The AUC reflects the total active drug concentration that reaches systemic circulation. The AUC in order of magnitude was bile > blood > kidney > liver > muscle. The results demonstrated that SMM in tilapia accumulated mainly in the bile.

The distribution of SMM in the blood and tissues (except in the bile) occurred from 1 to 24 h, and the drug concentrations were below or close to 100 µg kg^{-1} after 24 h. The relative distribution of SMM between plasma and tissues after oral administration is presented in Table 2. In the liver, the drug concentrations were higher than those in the plasma from 48 to 192 h, with liver to plasma ratios of 1.11—4.32. In the kidney, the drug concentrations were higher than those in the plasma from 120 to 192 h, with

Table 1 – The PK parameters of SMM and AC-SMM in blood and various tissues of tilapia.

PK parameter	Blood	Liver	Kidney	Bile	Muscle
SMM					
T_{max} (h)	1	1	1	15	1
C_{max} ($\mu g\ kg^{-1}$)	9.57×10^3	2.05×10^3	2.85×10^3	1.00×10^3	123
AUC ($h \cdot \mu g\ kg^{-1}$)	3.06×10^4	1.19×10^4	1.22×10^4	4.85×10^4	6.71×10^3
K_e (h^{-1})	0.032	0.028	0.027	0.015	0.007
$T_{1/2}$ (h)	21.7	24.8	25.7	46.2	99.0
AC-SMM					
C_{max} ($\mu g\ kg^{-1}$)	2.14×10^3	2.50×10^4	1.72×10^3	5.78×10^4	67.5
T_{max} (h)	1	1	4	24	1
AUC ($h \cdot \mu g\ kg^{-1}$)	2.52×10^4	1.14×10^5	2.08×10^4	4.06×10^6	8.70×10^3
Ke (h^{-1})	0.015	0.030	0.029	0.010	0.002
$T_{1/2}$ (h)	46.2	23.1	23.9	69.3	346.5

Data are expressed as mean value.

kidney to plasma ratios of 4.01–1.92. In the bile, the drug concentrations were higher than those in the plasma from 15 to 192 h, with bile to plasma ratios of 2.97–7.96. In the muscle, the drug concentrations were higher than those in the plasma from 96 to 192 h, with muscle to plasma ratios of 1.88–4.01. These results indicated that the distribution from blood to tissues was quite slow and the elimination from blood was more rapid than the distribution from tissues back to blood. The major pathway for elimination is from the liver to the bile to the intestine.

The k_e value reflects the ability of a drug to be excreted from the body. The k_e in order of magnitude was blood > liver > kidney > bile > muscle. In comparison, the k_e ($0.032\ h^{-1}$) of SMM in tilapia blood was higher than that in eel ($0.005\ h^{-1}$), yellowtail ($0.015\ h^{-1}$), and rainbow trout ($0.02\ h^{-1}$) [14]. $T_{1/2}$ reflects the elimination rate of a drug from the body. The $T_{1/2}$ of SMM in order of magnitude was muscle > bile > kidney > liver > blood. In tissue analyses, SMM elimination rates in the liver were more rapid because the liver is a well-perfused tissue a major metabolic organ and can quickly convert SMM into AC-SMM [8]. By contrast, SMM elimination rates in the muscle were the slowest because the muscle is a poorly-perfused organ, which may result in a longer elimination time. By comparing the $T_{1/2}$ of SMM in various fish species to that of tilapia, we revealed that the $T_{1/2}$

of SMM in tilapia blood (21.7 h) was shorter than that in eel (139 h) and rainbow trout (32.9 h), but longer than that in yellowtail (4.53 h) [14]. The ranges of the $T_{1/2}$ of SMM in the different fish are considerable and depend on many factors including species differences, age, sex, genetic predisposition, and environmental factors.

3.3. Metabolite analyses

The mean concentration of AC-SMM versus time in blood and tissues is illustrated in Fig. 4 and the PK parameters of AC-SMM are presented in Table 1. The AC-SMM blood concentrations increased rapidly from 0.5 to 1 h. C_{max} was reached at 1 h, and then the concentration decreased from 24 to 48 h. The redistribution of the drug occurred between 48 and 72 h. The final drug concentration at 192 h remained at 17.0 $\mu g\ kg^{-1}$. C_{max} of AC-SMM was reached at 1 (liver), 4 (kidney), 24 (bile), and l h (muscle). Notably, the bile had the highest drug concentration compared with the other tissues after 15 h. At 192 h, the drug concentration in the bile was far higher than that in the blood and other tissues.

Table 2 – Tissue/blood ratios of SMM after oral administration.

Time (h)	Liver/blood ratio	Kidney/blood ratio	Bile/blood ratio	Muscle/ blood ratio
0.5	0.15±0.04	0.46±0.12	0.19±0.03	0.10±0.01
0.75	0.07±0.02	0.16±0.02	0.12±0.02	0.05±0.03
1	0.21±0.01	0.30±0.02	0.04±0.00	0.01±0.00
4	0.37±0.01	0.28±0.04	0.56±0.19	0.08±0.03
8	0.25±0.03	0.32±0.02	0.91±0.09	0.10±0.01
15	0.26±0.04	0.22±0.07	2.97±0.18	0.15±0.06
24	0.69±0.15	0.52±0.11	3.18±0.50	0.27±0.11
48	1.11±0.22	0.54±0.08	5.34±2.95	0.38±0.06
72	0.81±0.12	0.49±0.08	6.50±5.11	0.57±0.02
96	1.19±0.27	0.88±0.23	21.77±6.54	1.88±0.49
120	1.16±0.05	4.01±1.56	20.43±5.45	1.48±0.35
144	1.12±0.56	3.18±1.11	13.56±4.02	2.44±1.27
168	3.12±0.72	2.51±0.93	10.39±3.19	4.36±0.56
192	4.32±2.12	1.92±1.12	7.96±6.36	4.01±0.86
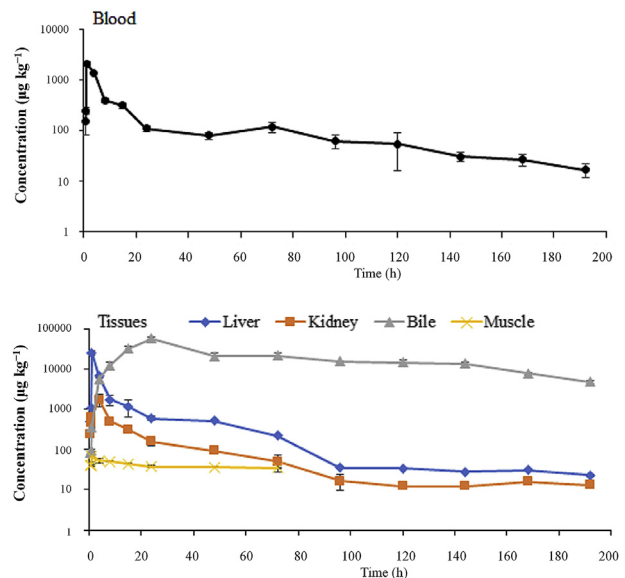
Fig. 4 – Semilogarithmic plot of blood and tissue concentration–time curves of AC-SMM.

The C_{max} of AC-SMM in order of magnitude was bile > liver > blood > kidney > muscle. The AUC of SMM in order of magnitude was bile > liver > blood > kidney > muscle. The AUC and C_{max} values were the highest in the bile. The results illustrated that high concentrations of AC-SMM accumulated in the bile. Biliary excretion, a process that is often facilitated by active transport systems located in the canalicular membrane of the hepatocyte, can be a crucial hepatic elimination pathway for many compounds [24]. The second highest level was found in the liver, which is instrumental in AC-SMM production.

Notably, the AC-SMM concentration was considerably higher in the internal organs, especially in the gallbladder. Kleinow, Beilfuss, Jorboe, Droy, and Lech [29] also reported a high concentration of AC-SMM in the bile of rainbow trout, which suggested the possibility of enterohepatic circulation. As a major excretion pathway in tilapia, bile excretion may result in the reabsorption of the drug. This cycling process is often associated with multiple plasma concentration peaks over time, and to some extent retards drug elimination [30]. In this study, the deviation from linearity at the 120 h time point in SMM blood (Fig. S8) indicated the possibility of reabsorption. These results were consistent with those in the above-mentioned studies.

Acetylation percentages were 45% (blood), 90% (liver), 62% (kidney), 98% (bile), and 52% (muscle). These findings indicate that acetylation was the main excretory metabolite in tilapia. Results of another study illustrated that SMM acetylation in the blood of eel, rainbow trout, and yellowtail following oral SMM administration was 3.8%, 49%, and 72%, respectively [14]. The differences in acetylation could be due to the differences in N-acetyltransferase activity among various fish [14].

The k_e values in order of magnitude were liver > kidney > blood > bile > muscle. The $T_{1/2}$ in order of magnitude was muscle > bile > blood > kidney > liver. Notably, the k_e value of AC-SMM ($0.015\ h^{-1}$) in the blood was lower than that of SMM ($0.032\ h^{-1}$). The plasma protein binding affinity of acetylated SMM was higher than that of SMM, which could affect their excretion rates [10,11].

According to the Taiwan Food and Drug Administration regulations [7], the residues of SMM drug are strictly regulated, but its metabolites are not included. The accumulation of N^4-metabolite in organisms may lead to nephrotoxicity [9,10]. If human unknowingly consume fish products containing these drug residues for a long period of time, it may endanger our health. This study showed that there was a large increase in the amount AC-SMM being accumulated in the liver and bile. Therefore, we suggest that the internal tissues of fish should be removed before consumption to minimize the risk. In addition, AC-SMM has a longer half-life than SMM in muscle. Consequently, the duration required for it to be excreted out of the muscles will be longer. As such, residues of AC-SMM drug in the muscle should also be of concern.

4. Conclusion

The LC–MS/MS method successfully determined trace levels of SMM and AC-SMM in fluid samples (blood and bile) and solid biological samples (liver, kidney, and muscle). PK analysis and tissue residues of SMM and AC-SMM in tilapia were reported in this study. SMM was rapidly absorbed and extensively distributed in tilapia. The results of the investigation of acetylation in blood confirmed that the N-acetyl reaction was the major metabolic pathway in tilapia. Regarding the drug excretion pathway, it was concluded that SMM and AC-SMM were excreted mainly through the biliary system in tilapia. Substantial concentrations of AC-SMM were still observed in tilapia bile. Therefore, it is suggested that the N-acetyl metabolite of SA should be included in the evaluation of drug residues. Performing SMM and AC-SMM PK studies in tilapia is necessary to ensure the correct use of SMM in this economically vital fish species in Taiwan.

This study demonstrated effective residue monitoring and investigated the PK of SMM and N^4-acetyl metabolites in tilapia.

Author contributions

Conception and design of study: Wei-Hsien Wang.

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Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfda.2018.08.007.

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