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Distribution and Elimination of Sulphadimethoxine and Its Metabolites in Treated Chicken

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Abstract—Sulphadimethoxine (SDM), and its metabolites, N_4-acetyl SDM, N_1-(2-methyl-6-hydroxy-4-pyrimidinyl) sulphanilamide (6-OH-SDM), N_1-(6-methyl-2-hydroxy-4-pyrimidinyl) sulphanilamide (2-OH-SDM), N_1-(2,6-dihydroxy-4-pyrimidinyl) sulphanilamide (2,6-diOH-SDM) and SDM N_1-glucuronide in chicken tissues were extracted, partially purified by Bond Elute SCX cartridges, and assayed and identified by HPLC/LC-MS after administration of SDM to chickens. During the administration and 24 h after withdrawal, SDM and 6-OH-SDM were observed in almost all tissues and excreta. N_4-Acetyl SDM and 2,6-diOH-SDM were observed in some tissues, but 2-OH-SDM and SDM N_1-glucuronide were observed in a few limited tissues. Twenty four hours after withdrawal, SDM and its metabolites, except 6-OH-SDM, decreased. SDM and its metabolites were eliminated from all tissues within 48 h of withdrawal.

Sulphadimethoxine, N_1-(2,6-dimethoxy-4-pyrimidinyl) sulphanilamide (SDM), has been widely used for prevention of poultry diseases by administration in feed (Ewing 1963; Morita & Akasi 1991). From the standpoint of public health, the residual antibacterials are not desirable (Takabatake 1981). In a previous study of the administration of 100 ppm SDM in feed, two days after withdrawal, residual SDM in chicken tissues was not observed (Nagata et al 1992). However, few reports have been published on residual SDM metabolites in chicken tissues. Metabolites of sulphadimethoxine have been found in the urine, plasma, serum or bile of man and a number of animals including rabbit, rat, pig, monkey, hen, cat, turtle, snail, dog, and rainbow trout. In chicken, the presence of N_4-acetyl SDM in urine (Adamson et al 1970; Takahasi 1986a) and tissue (Onodera et al 1970), SDM N_1-glucuronide in urine (Adamson et al 1970) and N_1-(2-methyl-6-hydroxy-4-pyrimidinyl) sulphanilamide (6-OH-SDM) in excreta (Takahasi 1986b) has been reported.

The kinetics of SDM and metabolites in chicken, during the administration and after withdrawal periods, have not been reported. The aim of this study was to investigate the metabolic fate of SDM in chicken tissues. In the present study, residual SDM and metabolites in tissues and excreta were examined by high-performance liquid chromatography (HPLC) and identified by thermospray liquid chromatography-mass spectrometry (LC-TSP-MS) after the administration of SDM to chickens.

Materials and Methods

Animals and SDM feeding studies

White Leghorn male chicks, 8-days old, donated by a local poultry farm, were fed with commercial feed for chicks for three weeks and then fed for a week with drug-free commercial feed for laying hens. Thereafter, the chickens were divided into two groups; one group received drug-free feed (control) and another group was fed with feed containing 100 mg kg^{-1} SDM. At day 19, from the beginning of the medication, three chickens in the medicated group were removed and placed on the drug-free diet. At day 20, three more chickens in the medicated group were removed and also placed on the drug-free diet. At day 21, all chickens were killed by decapitation, and liver, heart, lung, gallbladder, kidney, spleen, blood, breast muscle, thigh muscle, gizzard, glandular stomach, duodenal, caecum, rectum, skin, quill and vexillum were collected.

Both excreta and caecum contents were collected during the last two days before the end of the experiment in the control and the group medicated throughout. In animals with the changed diet, excreta and caecum contents were collected over the final 24 h. All these samples were stored at −20°C until analysis.

Chemicals and analytical methods

SDM was purchased from Sigma Chemical Company (St Louis, MO, USA) and N_4-acetyl SDM was synthesized according to the Japanese Pharmacopoeia (1971). (mp 214°C) (Found: C;47·70%, H; 4·57%, N;15·85%, $C_{14}H_{16}O_5N_4S$). SDM N_1-glucuronide was synthesized by a modification of the method of Bridges et al (1965). (mp 155–160°C (decomp.)) (Found: C; 38·78%, H; 5·63%, N; 12·33%, $C_{18}H_{25}N_5O_{10}S$ $3{\cdot}5H_2O$). N_1-(2,6-Dihydroxy-4-pyrimidinyl) sulphanilamide (2,6-diOH-SDM), 6-OH-SDM, and N_1-(6-methyl-2-hydroxy-4-pyrimidinyl) sulphanilamide (2-OH-SDM) were kindly donated by Mr Miura (Daiichi Seiyaku, Tokyo, Japan). Standard solutions of these drugs were prepared by dissolving each drug in a mixture of 0·01% ammonium solution and methanol (1:1, v/v). All other chemicals used were obtained commercially at the purest grade available. Bond Elute SCX (3 mL volume, Varian Sample Preparation Products, Harbor City, CA, USA) was preconditioned with 10 mL methanol followed by 10 mL water.

Isocratic HPLC determination. The liquid chromatograph consisted of a Model 980-PU pump equipped with a Model

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UV/vis-875 variable wavelength detector and a Model AS-875 automatic sampling system (Japan Spectroscopic Co., Ltd, Tokyo, Japan). A guard column, 4·6 mm i.d. × 10 mm, and an analytical column, 4·6 mm i.d. × 150 mm, packed with Inertsil C8 (Gaschro Kogyo Co., Inc., Tokyo, Japan), were used. Each 20-μL sample solution was injected into the HPLC by an automatic sampling system. The flow rate of each mobile phase was 1·0 mL min^{-1} and the column was maintained at 50°C. Other HPLC conditions were as follows. For SDM and N_4-acetyl SDM, the mobile phase was methanol-5 mM ammonium acetate (14:86, v/v) with detection at 268 nm. For 2,6-diOH-SDM and SDM N_1-glucuronide, the mobile phase was 5 mM ammonium acetate with detection at 260 nm. For 6-OH-SDM and 2-OH-SDM, the mobile phase was methanol-5 mM ammonium acetate (1:99, v/v) with detection at 280 nm.

LC-MS identification. A model 1090 HPLC was coupled to an HP 5989A mass spectrometer (Hewlett Packard) equipped with an HP TSP ion source with an HP-TSP-LC-MS interface (Yokogawa Analytical Systems, Co., Ltd, Tokyo, Japan). The analytical column was Excelpak SIL C18 5B (4·6 mm i.d. × 150 mm, Yokogawa Analytical Systems) maintained at 40°C with detection at 280 nm and flow rate of 1·0 mL min^{-1}. Other HPLC and MS conditions are described below.

For SDM and N_4-acetyl SDM, the mobile phase was methanol-5 mM ammonium acetate (20:80, v/v), the source temperature was 250°C, the stem temperature was 130°C, the repeller voltage was +100 V and ionization was conducted by both positive and negative filament modes. The MS was scanned from 150 to 450 m/z at 0·46 scans s^{-1}. Selected ion monitoring (SIM) was performed at m/z 311 for SDM and m/z 353 for N_4-acetyl SDM in a positive filament mode.

For 2,6-diOH-SDM and SDM N_1-glucuronide, the mobile phase was methanol-5 mM ammonium acetate (5:95, v/v), the source temperature was 250°C, the stem temperature was 130°C, the repeller voltage was +100 V and ionization was both positive and negative discharge modes. The MS was scanned from 150 to 550 m/z at 0·46 scans s^{-1}. SIM was conducted at m/z 283 for 2,6-diOH-SDM and m/z 311 for SDM N_1-glucuronide in the positive discharge mode.

For 6-OH-SDM and 2-OH-SDM, the mobile phase was methanol-5 mM ammonium acetate (5:95, v/v), the source temperature was 250°C, the stem temperature was 138°C, the repeller voltage was +100 V and ionization was both positive and negative discharge modes. The MS was scanned from 150 to 450 m/z at 0·46 scan s^{-1}. SIM was conducted at m/z 295 and m/z 297 for 6-OH-SDM and 2-OH-SDM, respectively, in positive and negative discharge modes.

All the LC-MS identification work was conducted at Yokogawa Analytical Systems.

Sample preparation

Quill, vexillum. Each sample was cut into small pieces. Quill (0·5 g) or vexillum (0·25 g) was accurately weighed and 1 mL 0·1% ammonium solution was added; the mixture was allowed to stand overnight. Other samples were cut into small pieces, 1 g of each sample was accurately weighed, 1 mL 0·01% ammonium solution was added, and then allowed to stand for a few hours.

Each sample in ammonium solution was homogenized for 1 min at moderate speed, mixed with 10 mL methanol, homogenized and centrifuged at 3000 rev min^{-1} for 10 min. The supernatant was filtered through cotton into a round bottom flask. Another 10 mL methanol was added to the residues, homogenized, centrifuged, and similarly filtered into the same flask. *n*-Propyl alcohol (10 mL) was added to the flask and concentrated to dryness under reduced pressure on a rotary evaporator at 50°C. The residue was dissolved with 2 mL methanol-water (1:1, v/v) in an ultrasonic bath for 1 min. The suspension was transferred to a centrifuge tube and centrifuged for 10 min at 3000 rev min^{-1}. The supernatant was placed into a sample vial for the automatic sampling system to detect SDM and N_4-acetyl SDM under the conditions described above.

Both methanol-water (1:1, v/v) solution in the sample vial and the centrifuge tube were collected together in the previous round bottom flask quantitatively by rinsing with methanol. *n*-Propyl alcohol (10 mL) was added, and then concentrated to dryness under reduced pressure on a rotary evaporator at 50°C. The residues were dissolved in 5 mL water in an ultrasonic bath for 1 min, transferred to a centrifuge tube, and then centrifuged for 10 min at 3000 rev min^{-1}. The supernatant was loaded onto the preconditioned Bond Elute SCX cartridge in vacuum at a flow rate of 1–1·2 mL min^{-1} and the washes were discarded. The cartridge was washed with two 2·5 mL portions of 0·1% ammonium water, the flask and the centrifuge tube were rinsed, and the solvent was passed through the cartridge. The eluate was collected for the determination of 2,6-diOH-SDM and SDM N_1-glucuronide. Two 2·5 mL portions of 0·2% of ammonium water were passed through the cartridge in succession and the eluate was collected. About 0·17 mL (1 drop) of acetic acid was mixed with the eluates, which were then applied to HPLC to determine 6-OH-SDM and 2-OH-SDM.

Results and Discussion

Feeding trial

During the administration period, the average feed intake of control and medicated groups was between 50 and 51 g day^{-1}/chicken and body weight gain was between 25 and 25·6 g day^{-1}/chicken. There was no difference between control and medicated groups.

Analytical studies

Extraction solvents. To solubilize the drugs bound to protein under stable conditions for the metabolites, 0·1 or 0·01% of ammonium water was used as an extraction solvent. The recoveries were similar when 0·1 and 0·01% ammonium solution was used as an extraction solvent. However, vexillum and quill were observed to be well homogenized when 0·1% ammonium solution was used. For the deproteinization, methanol was added to the 0·1 or 0·01% ammonium water extract. SDM and metabolites were stable for more than two days in 0·1% ammonium solution.

Table 1. Recovery of SDM and metabolites in tissues.

Tissue	Recovery (%)					
	SDM	N_4-Acetyl SDM	2,6-diOH SDM	SDM N_1-glucuronide	6-OH SDM	2-OH SDM
Liver	86·1	75·5	65·3	50·5	72·2	60·0
Heart	78·8	85·6	64·0	52·8	60·0	70·3
Lung	75·9	70·3	64·0	56·8	67·0	71·4
Kidney	95·9	97·7	80·8	54·6	71·4	62·6
Spleen	77·7	79·2	85·0	64·1	84·6	73·2
Blood	95·8	91·9	92·9	62·6	70·9	70·4
Breast muscle	81·7	80·5	68·6	69·9	62·8	80·3
Thigh muscle	80·1	76·2	78·0	49·6	70·2	72·7
Gizzard	87·2	81·9	67·4	52·2	62·5	84·9
Glandular stomach	90·8	80·0	70·0	62·7	84·4	81·9
Duodenal	84·0	86·9	99·8	50·4	59·7	65·8
Caecum	100·5	85·0	105·0	50·9	70·7	77·3
Skin	78·8	91·9	65·0	52·0	80·6	86·7
Vexillum	92·2	99·5	83·8	63·3	57·1	65·8

Recovery is the average of duplicate experiments. To each 1 g tissue was added 0·4 μg SDM, N_4-acetyl SDM, 2,6-diOH-SDM, 2 μg 6-OH-SDM, 2-OH-SDM and 20 μg SDM N_1-glucuronide. A quarter of the above quantities of each drug was added to 0·25 g vexillum.

Procedure. For SDM and N_4-acetyl SDM, the extract could be assayed directly by HPLC. Other metabolites needed preliminary purification of the extract using a Bond Elute SCX cartridge.

HPLC conditions. The UV absorption spectra of SDM, N_4-acetyl SDM, 2,6-diOH-SDM, SDM N_1-glucuronide, 6-OH-SDM and 2-OH-SDM in each mobile phase were constructed using a photo-diode array detector (Model MD-910, Japan Spectroscopic Co., Ltd). The maximum absorptions of SDM were observed at 203 and 268 nm, those of N_4-acetyl SDM were 203 and 267 nm. The detection wavelength was set at 268 nm. The maximum absorptions of 2,6-diOH-SDM were observed at 204, 260 and 280 nm, those of SDM N_1-glucuronide were 256 and 303 nm; compromise had to be made to obtain good sensitivities for both drugs, and the detection wavelength was set at 260 nm. The maximum absorptions of 6-OH-SDM were observed at 203 and 279 nm, those of 2-OH-SDM were 203, 263 and 287 nm; the detection wavelength was set at 280 nm.

Recoveries. The recovery tests were carried out by adding

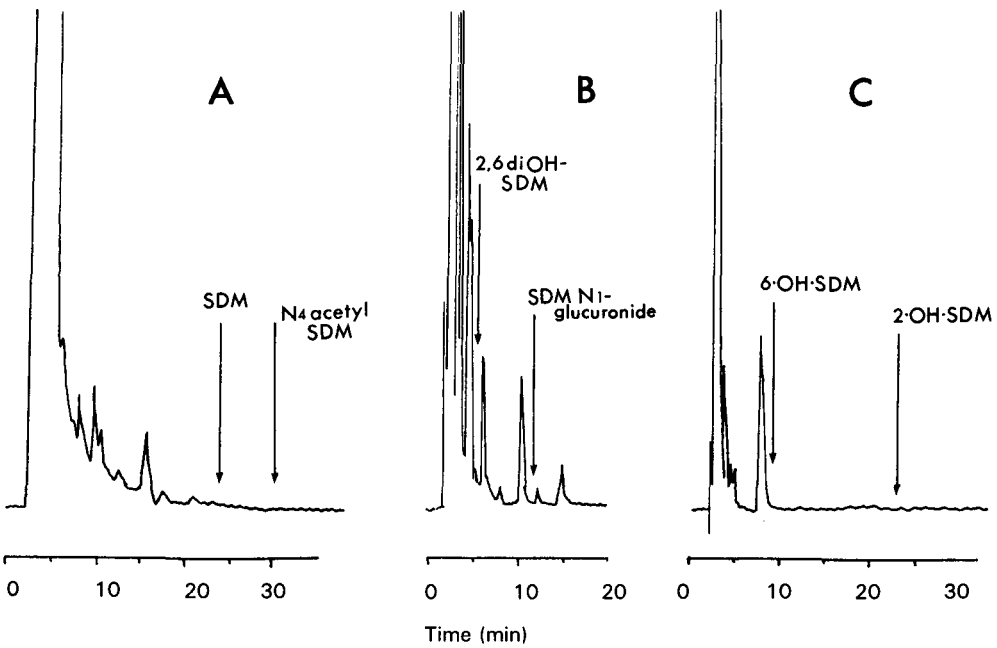

FIG. 1. HPLC chromatograms of sample extracts. A. Duodenal extract (non-medicated chicken). The retention times of SDM and N_4-acetyl SDM in standard solutions were 24·6 and 30·9 min, respectively. B. Liver extract (non-medicated chicken). The retention times of 2,6-diOH-SDM and SDM N_1-glucuronide in standard solutions were 5·6 and 11·5 min, respectively. C. Glandular stomach extract (non-medicated chicken). The retention times of 6-OH-SDM and 2-OH-SDM in standard solutions were 9·8 and 23·0 min, respectively.

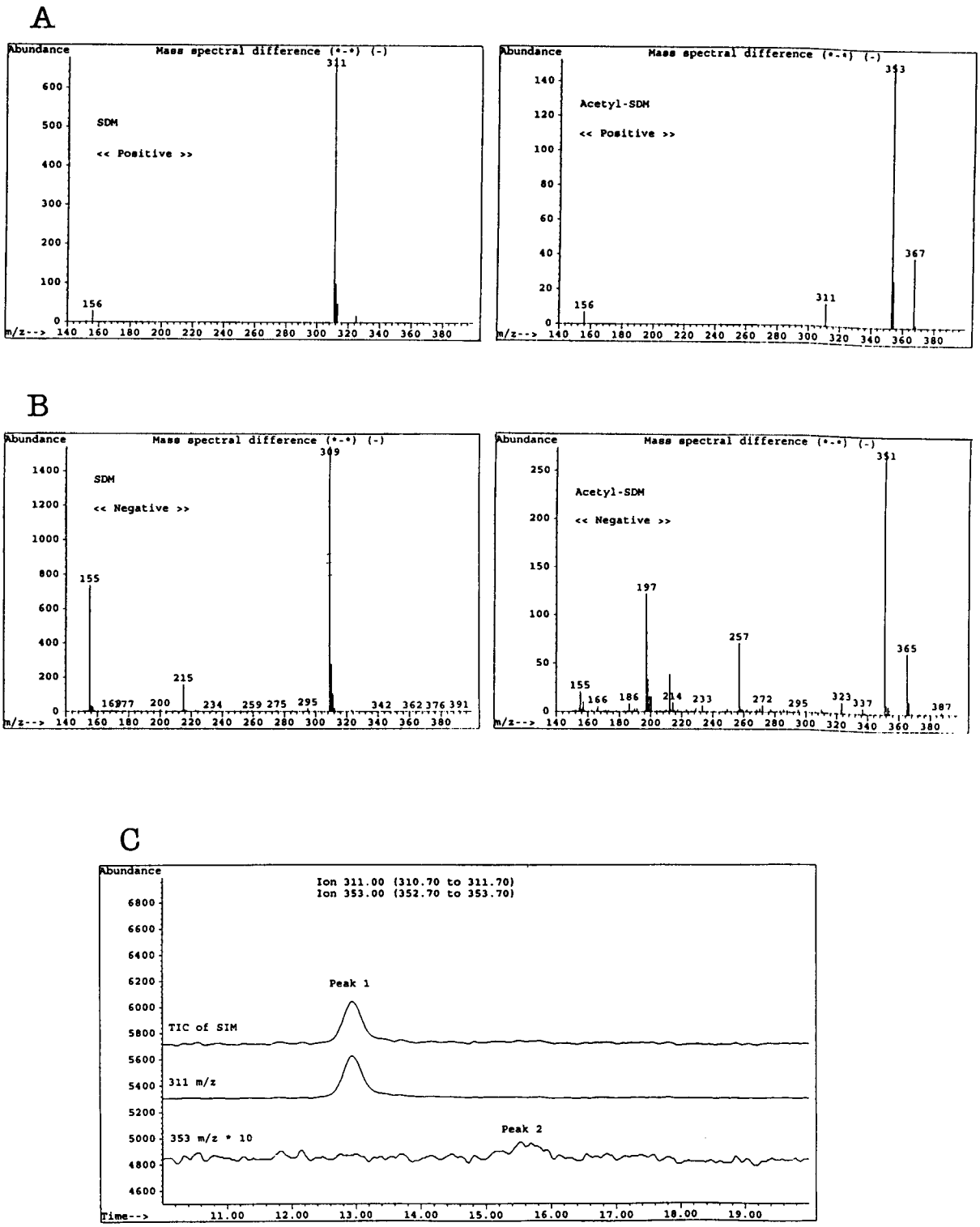

FIG. 2. Mass spectra of SDM (1) and N_4-acetyl SDM (2) standards in positive (A) and negative (B) modes and SIM of kidney extract in positive mode (C).

1 mL of the standard solution, which contained 0·4 μg mL^{-1} each of SDM, N_4-acetyl SDM and 2,6-diOH-SDM, 2 μg mL^{-1} each of 6-OH-SDM and 2-OH-SDM and 20 μg mL^{-1} SDM N_1-glucuronide (g tissue)$^{-1}$. To 0·5 g quill and 0·25 g vexillum, 1 mL of the half and the quarter concentrations of the standard mixture solution was added, respectively. These samples were allowed to stand for a few hours, and then the drugs were extracted by the method of sample preparation described above. The recovery rates of SDM and metabolites are shown in Table 1.

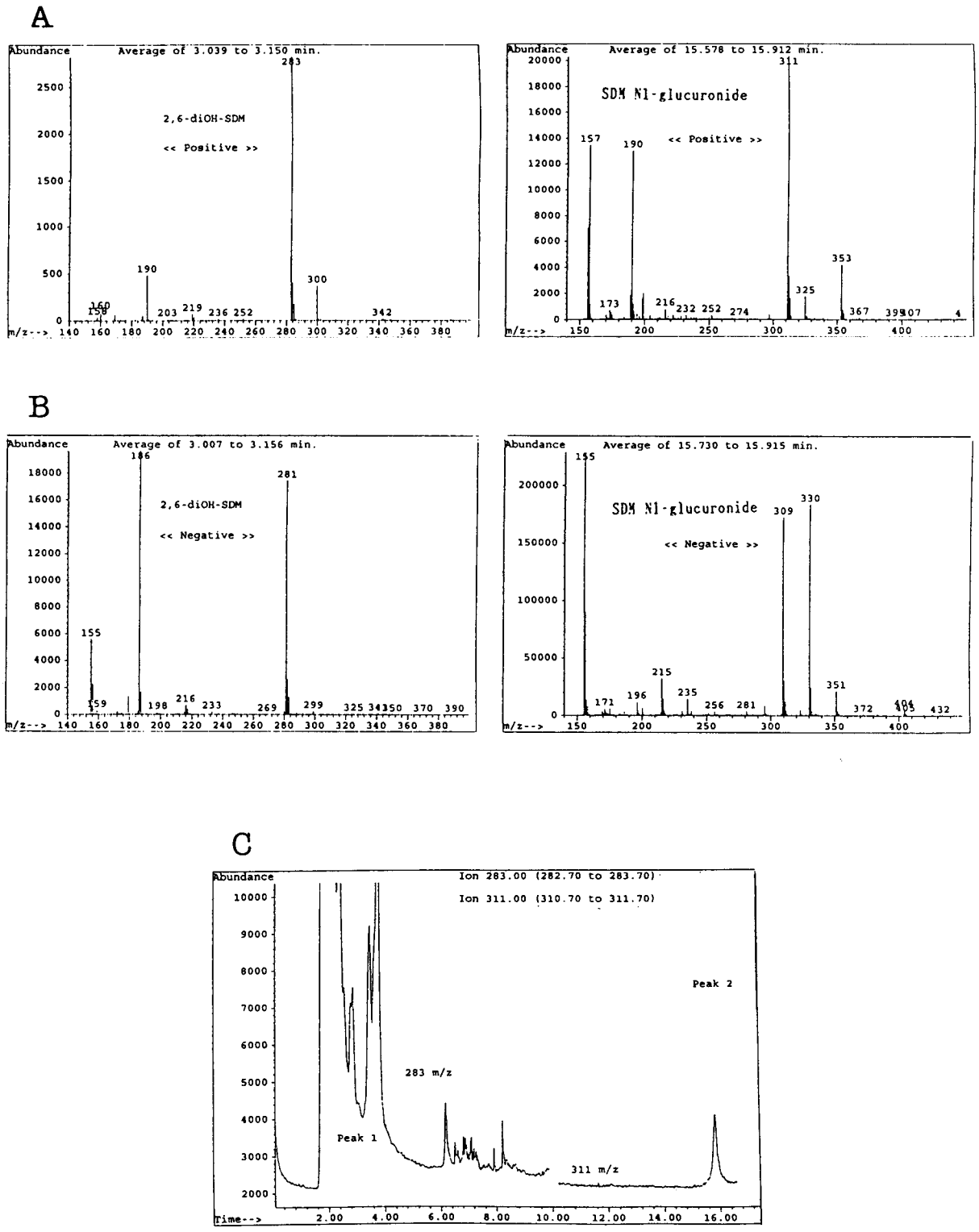

FIG. 3. Mass spectra of 2,6-diOH-SDM (1) and SDM N_1-glucuronide (2) standards in positive (A) and negative (B) modes and SIM of kidney extract in positive mode (C).

The recoveries of SDM N_1-glucuronide were poor in all tissues and fluctuation was observed in the recoveries of 2,6-diOH-SDM, SDM N_1-glucuronide and 6-OH-SDM. These inconsistencies might be partly due to interference by the endogenous materials of the sample extracts, elution of loaded 2,6-diOH-SDM and SDM N_1-glucuronide with water when the cartridge was washed, or adsorption of SDM N_1-glucuronide to the cartridge (Goto et al 1987).

Detection limits and calibration curves of SDM and meta-

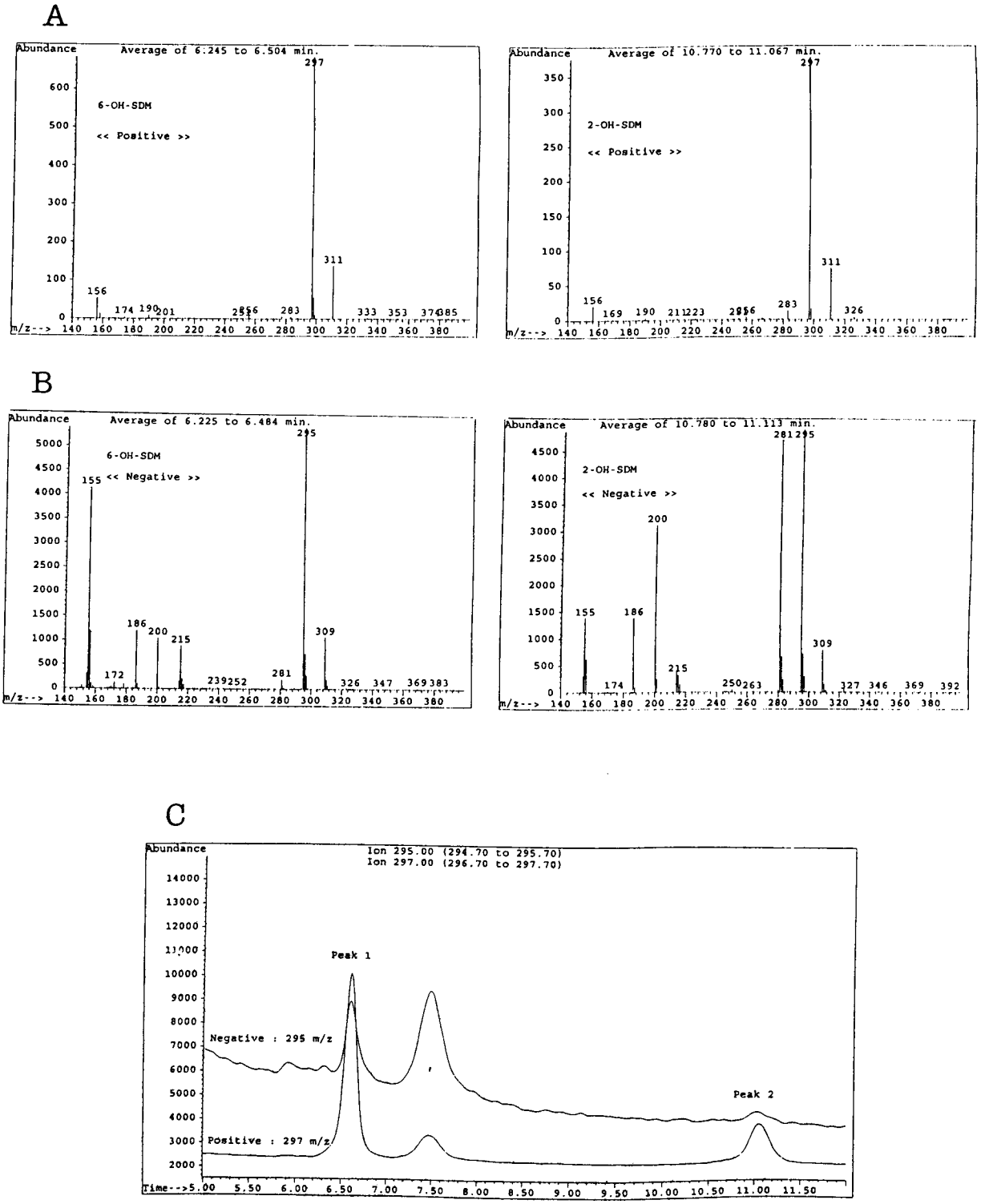

FIG. 4. Mass spectra of 6-OH-SDM (1) and 2-OH-SDM (2) standards in positive (A) and negative (B) modes and SIM of kidney extract in positive and negative modes (C).

bolites. The detection limits in tissues, defined as three times the background signal, were 0·05 $\mu g\,g^{-1}$ for SDM, N_4-acetyl SDM, 6-OH-SDM and 2,6-diOH-SDM, 0·1 $\mu g\,g^{-1}$ for 2-OH-SDM and 0·5 $\mu g\,g^{-1}$ for SDM N_1-glucuronide. In quill and vexillum, the quantitation limits of SDM, N_4-acetyl SDM, 6-OH-SDM and 2,6-OH-SDM were 0·1 and 0·2 $\mu g\,g^{-1}$, that of 2-OH-SDM was 0·2 and 0·4 $\mu g\,g^{-1}$, and that of SDM N_1-glucuronide was 1·0 and 2·0 $\mu g\,g^{-1}$, respectively.

The calibration curves of each standard were linear over the range of 0·05–3·0 $\mu g\,mL^{-1}$ for SDM and N_4-acetyl SDM, 0·02–2·0 $\mu g\,mL^{-1}$ for 2,6-diOH-SDM, 0·02–

Table 2. Residual SDM and metabolites in chicken tissues 24 h after withdrawal.

Tissue	Residual amount ($\mu g\,g^{-1}$)					
	SDM	N_4-Acetyl SDM	2,6-diOH SDM	SDM N_1-glucuronide	6-OH SDM	2-OH SDM
Liver	<0·05	<0·05	0·37 (0·55)	<0·5	1·08 (0·92)	0·36 (0·46)
Heart	<0·05	<0·05	0·15 (0·18)	<0·5	1·53 (1·25)	<0·1
Lung	0·05 (0·01)	<0·05	0·18 (0·23)	<0·5	0·80 (1·05)	<0·1
Gallbladder	0·19 (0·23)	0·28 (0·41)	2·83 (2·99)	<0·5	2·77 (0.71)	<0·1
Kidney	<0·05	<0·05	0·80 (0·67)	0·54 (0·06)	0·71 (1·13)	<0·1
Spleen	<0·05	<0·05	<0·05	0·51 (0·02)	0·17 (0·21)	<0·1
Blood	<0·05	<0·05	<0·05	<0·5	0·93 (1·03)	<0·1
Breast muscle	<0·05	<0·05	<0·05	<0·5	1·20 (1·17)	<0·1
Thigh muscle	<0·05	<0·05	<0·05	<0·5	0·85 (1·18)	<0·1
Gizzard	0·06 (0·02)	<0·05	0·33 (0·49)	<0·5	1·25 (1·12)	<0·1
Glandular stomach	<0·05	<0·05	0·22 (0·28)	<0·5	0·77 (1·23)	<0·1
Duodenal	<0·05	<0·05	0·76 (1·24)	0·59 (0·15)	2·77 (1·82)	<0·1
Ceacum	0·13 (0·15)	0·25 (0·36)	<0·05	2·13 (2·83)	1·09 (1·12)	<0·1
Rectum	0·13 (0·16)	<0·05	<0·05	<0·5	0·26 (0·35)	<0·1
Skin	0·09 (0·03)	<0·05	<0·05	<0·5	0·70 (1·12)	<0·1
Quill	0·13 (0·07)	<0·1	<0·1	<0·2	3·39 (1·64)	<0·2
Vexillum	2·37 (0·47)	<0·2	<0·2	<0·5	<0·21	<0·4

Each value represents the mean (s.d.) of three chickens.

1·0 $\mu g\,mL^{-1}$ for 6-OH-SDM, 0·04–2·0 $\mu g\,mL^{-1}$ for 2-OH-SDM and 0·2–5·0 $\mu g\,mL^{-1}$ for SDM N_1-glucuronide. HPLC chromatograms of sample extracts are shown in Fig. 1.

LC-MS identification. The MS spectra of SDM, N_4-acetyl SDM, 2,6-diOH-SDM, SDM N_1-glucuronide, 6-OH-SDM, 2-OH-SDM and selected-ion monitoring chromatograms of kidney extracts are shown in Figs 2–4.

There were little or no fragments in the mass spectra of SDM and metabolites. In the specta of SDM (mol. wt 310) and N_4-acetyl SDM (mol. wt 352), protonated molecular ions, m/z 311 and m/z 353, deprotonated molecular ions, m/z 309 and m/z 351, were observed in positive and negative modes, respectively. In the spectra of 6-OH-SDM and 2-OH-SDM (mol. wt 296), protonated molecular ion, m/z 297, and deprotonated molecular ion, m/z 295, were observed in positive and negative modes. The peak with retention time 7·5 min, which was not observed in blank tissue, could not be identified. In the spectra of 2,6-diOH-SDM (mol. wt 282), protonated molecular ion, m/z 283, and deprotonated molecular ion, m/z 281, were observed in positive and negative modes, respectively. In the spectra of SDM N_1-glucuronide, the molecular ion was not observed, but m/z 311 and m/z 309, which are assigned to protonated and deprotonated SDM were observed. The detection and identification of SDM and metabolites in sample extracts, were performed by SIM.

Distribution of SDM and metabolites in chicken tissues and excreta. As was expected, in the control group, neither SDM nor metabolites were found in tissues and excreta.

Table 3. Residual SDM and metabolites in medicated-chicken tissues.

Tissue	Residual amount ($\mu g\,g^{-1}$)					
	SDM	N_4-Acetyl SDM	2,6-diOH SDM	SDM N_1-glucuronide	6-OH SDM	2-OH SDM
Liver	0·17 (0·03)	<0·05	0.28 (0·40)	<0·05	<0·05	<0·1
Heart	0·25 (0·12)	<0·05	<0·05	<0·05	0·18 (0·23)	<0·1
Lung	0·26 (0·05)	0·07 (0·05)	0·35 (0·27)	<0·05	<0·05	<0·1
Gallbladder	0·13 (0·13)	0·22 (0·30)	0·19 (0·24)	0·62 (0·24)	0·20 (0·26)	<0·1
Kidney	0·99 (0·27)	0·06 (0·02)	1·57 (1·93)	1·84 (2·13)	0·53 (0·46)	0·18 (0·23)
Spleen	0·25 (0·25)	0·08 (0·04)	<0·05	<0·05	0·47 (0·38)	<0·1
Blood	0·66 (0·36)	<0·05	0·71 (1·15)	<0·05	0·32 (0·47)	<0·1
Breast muscle	0·08 (0·03)	<0·05	<0·05	<0·05	<0·05	<0·1
Thigh muscle	0·10 (0·04)	<0·05	<0·05	<0·05	<0·05	<0·1
Gizzard	0·34 (0·33)	<0·05	0·11 (0·08)	0·60 (0·23)	0·18 (0·22)	<0·1
Glandular stomach	0·12 (0·04)	<0·05	<0·05	<0·05	0·17 (0·21)	<0·1
Duodenal	0·19 (0·02)	0·05 (0·01)	<0·05	0·60 (0·11)	0·81 (1·32)	<0·1
Caecum	0·76 (0·82)	0·14 (0·11)	0·21 (0·29)	<0·05	0·53 (0·45)	0·42 (0·32)
Rectum	0·07 (0·03)	<0·05	<0·05	<0·05	0·13 (0·13)	<0·1
Skin	0·15 (0·01)	0·07 (0·03)	<0·05	<0·05	<0·05	<0·1
Quill	0·31 (0·07)	<0·1	<0·1	<0·2	0·84 (0·36)	<0·2
Vexillum	1·99 (0·78)	<0·2	0·57 (0·90)	<0·4	<0·2	<0·4

Each value represents the mean (s.d.) of three chickens.

Table 4. Residual SDM and metabolites in excreta.

	Residual amount ($\mu g\,g^{-1}$)					
	SDM	N_4-Acetyl SDM	2,6-diOH SDM	SDM N_1-glucuronide	6-OH SDM	2-OH SDM
Excreta						
A (24–48 h)	1·76	nd	0·06	<0·5	1·40	<0·1
B (0–24 h)	7·93	nd	0·11	<0·5	1·90	<0·1
C (medication)	30·4	3·81	1·38	<0·5	3·80	0·21
Caecum contents						
A (24–48 h)	0·05	nd	0·31	<0·5	<0·05	<0·1
B (0–24 h)	2·11	nd	0·94	<0·5	0·46	<0·1
C (medication)	19·5	3·97	1·40	<0·5	2·60	0·28

Each concentration is the average of two trials. A (24–48 h): excreta and caecum contents between 24 and 48 h after withdrawal. B (0–24 h): excreta and caecum contents during 24 h after withdrawal. C (medication): excreta and caecum contents of the group medicated throughout during the last two days before the experiment.

In the group where the chickens were fed with drug-free feed for 48 h after withdrawal, residual SDM and metabolites in tissues were not found, except for 1·27–1·34 $\mu g\,g^{-1}$ SDM in vexillum.

Residual SDM and metabolites in tissues in the group where the chickens were fed drug-free feed for 24 h after withdrawal, and in the group medicated throughout, are shown in Tables 2 and 3, respectively. Residual SDM and metabolites in the excreta and caecum contents are shown in Table 4.

There was considerable fluctuation in the concentrations of SDM and metabolites in tissues by individual chickens. These variations are assumed to reflect partly the inconsistency of the eating habits of individual chickens and the rapidity of excretion of SDM and metabolites.

SDM. During the administration period, relatively high concentrations of SDM were found in blood, kidney, caecum and vexillum. Although high concentrations of residual SDM in blood and kidney had been already reported (Onodera et al 1970; Yamamoto et al 1979; Takahasi et al 1991), those in caecum and vexillum had not been reported. Because the wall of the rectum and cloaca have villi and circular folds, which are similar to those structures of the intestine (Foust 1952), water is absorbed from urine during its passage from the cloaca through the colon to the caecum by anti-peristal contraction (Duke 1977; Vree & Hekster 1985) and the drugs are presumed to be absorbed simultaneously with the water in the caecum, and to result in the high concentration of the drugs.

Yamamoto et al (1979) and Takahashi et al (1991), who administered drinking water containing 0·4% or 1000 mg kg^{-1} SDM, observed residual SDM in the skin up to 10 or 5 days after withdrawal, longer than the time noted for residues of SDM in the kidney. In the present study, 0·16 $\mu g\,g^{-1}$ residual SDM was observed in the skin, but 48 h after withdrawal residual SDM was not observed. These differences may arise because SDM in skin has already been transferred through the quill to the vexillum, since feathers are epidermal structures, and in that respect similar to hair (Foust 1952).

During the administration period, the high concentrations of SDM excreted may be due to unabsorbed SDM or from hydrolysed conjugates in the gut (Bridges et al 1968; Adamson et al 1970).

Metabolites. In the present study, N_4-acetyl SDM is not the main metabolite of SDM; during the administration period, this compound was observed in a few tissues and in excreta in limited concentrations; moreover, it decreased rapidly from excreta after withdrawal. Onodera et al (1970) reported that N_4-acetyl SDM was found in all tissues of chickens; however, acetylation and deacetylation is known to take place in tissues of many species (Vree et al 1983, 1984, 1989; Vree & Hekster 1985, 1987). Shaffer & Bieter (1950) observed that when acetyl sulphonamides were administered to chickens, 70·3–100% free sulphonamides were excreted in urine and acetylated SDM was deacetylated in-vitro in kidney and liver. In birds, the deacetylation process has been reported to be substantial (Vree & Hekster 1985) and acetylation of sulphonamides is not estimated to be the predominant pathway of metabolism.

6-OH-SDM was the main metabolite of SDM in the present study; 24 h after withdrawal, the concentrations of 6-OH-SDM increased, except in spleen, and the depletion of it in excreta was observed to be slow.

The residual 2-OH-SDM and 2,6-diOH-SDM in chicken tissues and excreta have not previously been reported. It is considered that 6-OH-SDM and 2-OH-SDM, which was not observed in excreta, were further hydroxylated to 2,6-diOH-SDM.

In chickens, SDM N_1-glucuronide is a minor metabolite (Adamson et al 1970; Vree & Hekster 1985). In the present study, SDM N_1-glucuronide was mainly found in gall-bladder, kidney, duodenal tissue and caecum, during the administration and 24 h after withdrawal. Glucuronides have been reported in urine and plasma, but seldom in tissues. Neither in excreta nor in caecum contents, was SDM N_1-glucuronide found during the experimental period. SDM N_1-glucuronide was assumed to be degraded in the gut (Bridges et al 1968; Adamson et al 1970) or that birds do not form SDM N_1-glucuronides. There may be no need for glucuronidation, because the pH of the excreta is 8–9, enabling a high renal excretion. In man, urine pH of 5–6 favours tubular reabsorption and low renal clearance (Vree & Hekster 1985, 1987). Further investigation is considered to be necessary to verify the existence of SDM N_1-glucuronide in chicken tissues.

In birds, it has been considered that acetylation of sulphonamides is low, while hydroxylation, which is not followed by glucuronidation, is high (Vree & Hekster 1985). SDM in chicken is assumed to be hydroxylated mainly to 6-OH-SDM and slightly to 2-OH-SDM, and thereafter, further hydroxylated to 2,6-diOH-SDM.

Acetylated metabolites have no antibacterial activities, but hydroxylated metabolites of SDM still have a *p*-aminophenyl group, which has antibacterial activity (Nouws et al 1985). However, their antimicrobial activities are considered to be less than 40% that of the parent drug and moreover they are eliminated from tissues within 48 h of withdrawal; the pharmacological effects of residual metabolites seems to be low. We conclude that a waiting time of 2–3 days after SDM administration is necessary before slaughtering the chickens for consumption.

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