J Korean Soc Appl Biol Chem (2014) 57(3), 397–405
DOI 10.1007/s13765-014-4027-y

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

In vitro Metabolism of Flucetosulfuron by Artificial Gastrointestinal Juices

Yong-Sang Lee · Joon-Kwan Moon · Kwang-Hyeon Liu · Eunhye Kim · Hoon Choi · Jeong-Han Kim

Received: 23 January 2014 / Accepted: 8 May 2014 / Published Online: 30 June 2014
© The Korean Society for Applied Biological Chemistry and Springer 2014

Abstract To investigate the metabolism of pesticides by gastrointestinal (GI) juices, artificial GI juices were incubated with threo- and erythro-isomers of flucetosulfuron. The metabolites produced in each reaction mixture of artificial GI juices were unambiguously identified using liquid chromatography-tandem mass spectrometry. Flucetosulfuron was observed to be stable in saliva. However, in the intestinal juices, approximately 18% of flucetosulfuron was degraded, producing N-(4,6-dimethoxypyrimidin-2-ylcarbomoyl)-2-(2-fluoro-1-hydroxypropyl)pyrimidine-3-sulfonamid (M1). In artificial gastric juices, about 85% of flucetosulfuron was rapidly degraded, producing the metabolites 2-(2-fluoro-1-hydroxypropyl) pyridine-3-sulfonamide (M2), 4,6-dimethoxypyrimidin-2-amine (M3), and 2-fluoro-1-(3-sulfamoylpyridin-2-yl)propyl 2-methoxyacetate (M4). These results indicate that the sulfonylurea bridge and ester bond of flucetosulfuron are hydrolyzed in artificial GI juices. No significant differences were noted in the degradation patterns between the two isomers of flucetosulfuron in the artificial GI juices that were tested. Considering the rapid degradation of flucetosulfuron *in vitro* by artificial GI juices, it is likely that there would be no significant absorption of flucetosulfuron from the GI tract into the blood stream after oral administration.

Keywords flucetosulfuron · gastrointestinal juice · metabolism · sulfonylurea

Y.-S. Lee · E. Kim · J.-H. Kim (✉)
Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Republic of Korea
E-mail: kjh2404@snu.ac.kr

J.-K. Moon
School of Plant, Life and Environmental Sciences, Hankyong National University, Ansung 456-749, Republic of Korea

K.-H. Liu
College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea

H. Choi
Ministry of Food and Drug Safety, Cheongwon 363-951, Republic of Korea

Y.-S. Lee
LG Life Sciences, Onsan-eup, Ulsan, 689-896, Republic of Korea

Introduction

In an effort to protect humans from the adverse effects of pesticides, tests of acute toxicity, carcinogenicity, and chronic toxicity in mammals are mandatory for the local registration of pesticides. These studies are evaluated by international scientific experts to gauge risk to humans and determine the acceptable daily intake level and maximum residue limit for the pesticides. Furthermore, to minimize the amount of pesticides in crops and to ensure that the residue level is below allowable levels, the pre-harvest interval for applying pesticides is recommended to farmers and pesticide applicators. According to a recent risk assessment of human safety in Korea, the current level of intake of pesticides through food is estimated to be very low (Lee et al., 2010; Yang et al., 2012).

Nevertheless, pesticide poisoning cases occur frequently. The most frequent cause of pesticide poisoning is not through food or occupational intake, but by intentional oral ingestion of pesticides during suicide attempts (Jei, 1992; Lee, 2010; Chang et al., 2012; Roh, 2012; Zhang and Li, 2012). As a result, countries such as Finland, Sri Lanka, and Taiwan have banned the local use of highly toxic pesticides. This has resulted in reductions in the number of suicides because of pesticide ingestion (Chang et al., 2012). In order to adopt a similar plan in Korea, paraquat, a highly toxic pesticide frequently used in suicide attempts was banned by the Rural Development Administration (RDA) in 2013, despite its overwhelming market share in Korea. As a preventive measure, the legislation bans the sale of highly toxic pesticides; however, such measures have not been undertaken worldwide. Therefore, safety assessment of each pesticide is required.

Recent studies have focused on the *in vitro* metabolism of pesticides by human liver microsomes (HLMs). These studies reveal the metabolic patterns and metabolizing enzymes in human

liver, allowing for the calculation of hepatic clearance and the detoxifying action of the liver. This enables a realistic risk assessment of pesticide toxicity (Lee et al., 2006; Abass et al., 2012). Human liver microsomal studies are limited to the metabolic processing of pesticides in the liver and do not provide any information on absorption from skin or gastrointestinal (GI) tract into the circulatory system. Thus, to thoroughly understand the metabolism after oral ingestion of pesticides, metabolism studies of pesticides by the GI tract in humans are also required. GI metabolism includes digestion by saliva, gastric juice, and intestinal juice.

Compared to *in vivo* studies, *in vitro* techniques can save labor and time, reduce cost, and improve accuracy and reproducibility while avoiding the ethical problems of human studies. A number of *in vitro* GI tract models are currently available for nutrition, toxicology, pharmacology, and safety assessments. A well-designed *in vitro* model may provide an accurate estimate of what is occurring *in vivo*; however, the correlation between *in vitro* and *in vivo* studies is highly dependent on model design and the physical and chemical properties of the materials tested. Therefore, before the *in vitro* model is applied, a quantitative validation of the model is recommended to estimate the *in vivo* situation (Versantvoort et al., 2005).

When secreted into the stomach, gastric juices have a pH =2, but the acidity may be decreased by dilution with food. Studies on the relationship between drug absorption and food digestive conditions have shown that food intake influences the bioavailability of some drugs (Melander, 1978; Weitschies et al., 2005). Abrahamsson et al. (2004) reported that food could significantly delay drug tablet disintegration in the stomach by formation of a film around the tablet.

As reported the unintentional ingestion quantity of soil by children in China can reach to 200 mg per day, thus, recent studies on mobilization of organochlorine pesticides (OCPs) and hydrophobic organochlorine pesticides in contaminated soils were carried out through an *in vitro* GI model (Tao et al., 2009; Tao et al., 2010). The study revealed that contaminated pesticides could be extracted by an *in vitro* GI model, providing a clue that the bound residue of OCPs could be mobilized. Adenugba et al. (2008) accurately described the composition of artificial GI juices. The compositions of GI juices can be modified according to the purpose of the study, such as digestion pattern of chemicals, stability of coating material for yogurt bacteria, availability of bound residual pesticides, or absorption of drugs (Rao et al., 1989; Gorbach et al., 1992; Yang et al., 1993; Adenugba et al., 2008; Virkel et al., 2009; Hur et al., 2011).

Flucetosulfuron, a selective herbicide developed in Korea by LG Life Sciences Ltd. (formerly LG Chem. Ltd., Korea) was tested in this study. Since this pesticide was developed as a diastereomer, there may be a different metabolic pattern for each isomer.

This study was carried out to understand the stability and disintegration patterns of flucetosulfuron and to provide information for the human risk assessment of flucetosulfuron by investigating the metabolism in artificial GI juices and identification of metabolites. On the basis of the metabolism by artificial GI juices, a relevant metabolic pathway for flucetosulfuron is proposed.

This study on the *in vitro* metabolism of flucetosulfuron by artificial GI juices should aid our general understanding of the disintegration and absorption of pesticides after ingestion and show the stability of the pesticide in each digestive organ of the human body.

Materials and Methods

Chemicals and Reagents. Flucetosulfuron isomers, threo-flucetosulfuron and erythro-flucetosulfuron, were isolated and purified by preparative- high performance liquid chromatography (HPLC) method. Threo-metabolite-1 (threo-M1 or TM1) and erythro-metabolite-1 (erythro-M1 or EM1) were acquired by hydrolysis of each purified flucetosulfuron isomer (Fig. 1). 2-(2-Fluoro-1-hydroxypropyl)pyridine-3-sulfonamide (M2) and 2-fluoro-1-(3-sulfamoylpyridin-2-yl)propyl 2-methoxyacetate (M4), having both threo- and erythro-isomers, were provided by LG Life Sciences Ltd (Korea). 4,6-Dimethoxypyrimidin-2-amine (M3) was purchased from Sigma-Aldrich (USA).

Ammonium acetate, acetic acid, and formic acid (above 99.9%) for HPLC and LC-MS/MS were purchased from Sigma-Aldrich. HPLC-grade acetonitrile and methanol were purchased from Burdick and Jackson (USA). α-Amylase, bile, mucin, pancreatin, pepsin, trypsin, urea, and uric acid for the preparation of artificial GI juices were products of Sigma-aldrich. Calcium chloride ($CaCl_2$), sodium chloride (NaCl), sodium isocyanide (NaSCN), sodium hydrogen carbonate ($NaHCO_3$), sodium sulfate (Na_2SO_4), and potassium chloride (KCl) for the preparation of buffer solution or artificial GI juices were the products of Daejung Chem (Korea). Potassium phosphate monobasic/dibasic and sodium hydroxide (NaOH) were purchased from Daejung Chem. The solvents and other chemicals used in the present study were of HPLC grade purchased from Burdick and Jackson (USA).

Instrument and conditions. HPLC, composed of Waters alliance 2690 HPLC and UV 4890 detector (Waters, USA) was used to analyze flucetosulfuron isomers and metabolites. The concentrations of flucetosulfuron isomers and its metabolites (TM1 and EM1) were measured by Waters alliance 2690 HPLC equipped with a Capcell pak C18 UG120 column (4.6 mm i.d. ×150 mm, 3 μm; Shiseido, Japan) at 40°C. The mobile phases consisted of 20 mM ammonium acetate buffer containing 0.1 M acetic acid (A) and acetonitrile (B). The gradient condition was as follows: 20% B at 0 min, 27% B at 4 min, 30% B at 25 min, and 20% B at 28–33 min. The injection volume was 10 μL, and peaks were detected at the wavelength of 254 nm.

LC-MS/MS, a tandem mass quadruple mass spectrometer (API2000 LC-MS/MS, Applied Biosystems, USA) coupled with an Agilent 1100 series HPLC system (Agilent, USA) was used

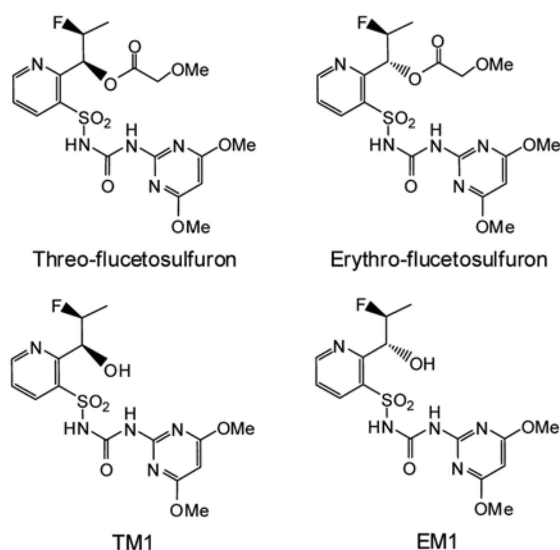

Fig. 1 Structures of flucetosulfuron and its metabolites.

with a Capcell pak C18 UG120 column (4.6 mm i.d. ×150 mm, 3 mm; Shiseido, Japan). The mobile phase consisted of 10 mM ammonium acetate containing 0.1% formic acid (A) and acetonitrile (B) at a flow rate of 1.0 mL/min. The gradient condition was as follows: 20% B at 0 min, 27% B at 4 min, 30% B at 25 min, and 20% B at 28–33 min. The injection volume was 2 μL. Mass spectra were recorded by electrospray ionization with a positive mode. The turbo ion spray interface was operated at 4500 V and 550°C. The operating conditions were optimized by flow injection of analytes and were determined as follows: nebulizer gas flow, 50 psi; curtain gas flow, 10 psi; and collision energy, 30 eV. Quadrupoles Q1 and Q3 were set on unit resolution. Centrifuge, a Hanil Micro17TR model having temperature control system, manufactured by Hanil Ltd (Korea), was used to acquire the supernatant of reaction samples.

Preparation of artificial GI juices. Phosphate buffer at 50 mM (pH 7.4) was used as neutral control. As acidic control, pH 2 buffer solution was prepared by adjusting the pH of 50 mM phosphate buffer to 2.0 with 6 N HCl. Each composition of artificial GI solutions was weighed and solubilized to 1 L volume (Table 1). The artificial saliva and intestinal juices were adjusted to pH 7.4 with $NaHCO_3$, and artificial gastric juice was adjusted to 2.0 with 6 N HCl. The pH adjusted GI solutions were finally made up to total volume (100%). The mixture of saliva + gastric juice (1:4 *v/v*) was prepared by mixing artificial saliva and gastric juice with a ratio of 1:4. All solutions were stored at 4°C, and used within 24 h. Buffer solutions at pH 7.4 and 2 were tested as controls.

***In vitro* metabolism of flucetosulfuron by GI juices.** The concentration of flucetosulfuron in each GI solution (neutral control, acidic control, saliva, saliva + gastric juice (1:4 *v/v*), gastric juice, intestinal juice) was 100 M and final volume of each

Table 1 Composition of artificial gastrointestinal juices (Adenugba et al., 2008)

Artificial saliva	mg/L	Artificial gastric juice	mg/L	Artificial intestinal juice	mg/L
NaCl	500	NaCl	2900	KCl	300
NaSCN	150	KCl	7000	$CaCl_2$	500
Na_2SO_4	550	KH_2PO_4	2700	$MgCl_2$	200
$NaHCO_3$	150	Pepsin	1000	$NaHCO_3$	1000
KCl	450	Mucin	3000	Trypsin	300
KH_2PO_4	600			Pancreatin	9000
$CaCl_2 \cdot 2H_2O$	150			Bile	9000
Mucin	750			Urea	300
α-amylase	250				
Urea	100				
Uric acid	10				

GI juice was adjusted to 250 μL. The metabolic reaction was carried out in a shaking water bath with 80 cycle/min at 37°C. The times for each metabolic reaction were 5 min for saliva, 2 h for acidic control, saliva + gastric juice (1:4 *v/v*) and gastric juice, and 6 h for neutral control and intestinal juice. After metabolic reaction for each designed time, the reaction samples were terminated by addition of 250 μL acetonitrile. After immediately vortexing for 1 min, the samples were centrifuged at 10,770×*g* for 5 min. Ten microliters of each supernatant of reaction mixtures were immediately analyzed by HPLC/UVD. The metabolic reaction was carried out in triplicate.

Identification of metabolites. Each 2 μL supernatant of saliva + gastric juice (1:4 *v/v*), gastric juice, intestinal juice were also analyzed by LC-MS/MS to identify the structure of the unknown metabolites. LC-MS/MS fragmentation patterns based on the mass value $[M+H]^+$ of LC-MS/MS were analyzed, and the candidate structure of metabolites were drawn. In addition, tandem mass spectrometry (MS/MS) analyses of TM1, EM1, M2, M3. M4, and the parent flucetosulfuron isomers were conducted to compare their fragmentation patterns. Each solution of the metabolites and parent flucetosulfuron isomers were prepared in acetonitrile at 10 ppm level, and the corresponding metabolic reaction supernatant was analyzed using HPLC for confirmation.

Quantification of flucetosulfuron and metabolites. After identification of metabolites, quantitative analysis was performed with an external standard calibration method. Standard solutions of threo-flucetosulfuron, erythro-flucetosulfuron, TM1, and EM1 were prepared as stock solutions with 1 mM in acetonitrile. The stock solution was diluted at the concentrations of 0.2, 0.5, 1.0, 10, and 100 μM with 1 mM in acetonitrile. Concentrations of flucetosulfuron and metabolites were obtained based on the peak area from each calibration standard. The calibration standards were fitted with high linearity (r^2 >0.999). Standard solutions of M2, M3, and M4 were also prepared as stock with 100 μM acetonitrile. The stock was diluted at the concentration of 0.5, 1.0, 10, and 100

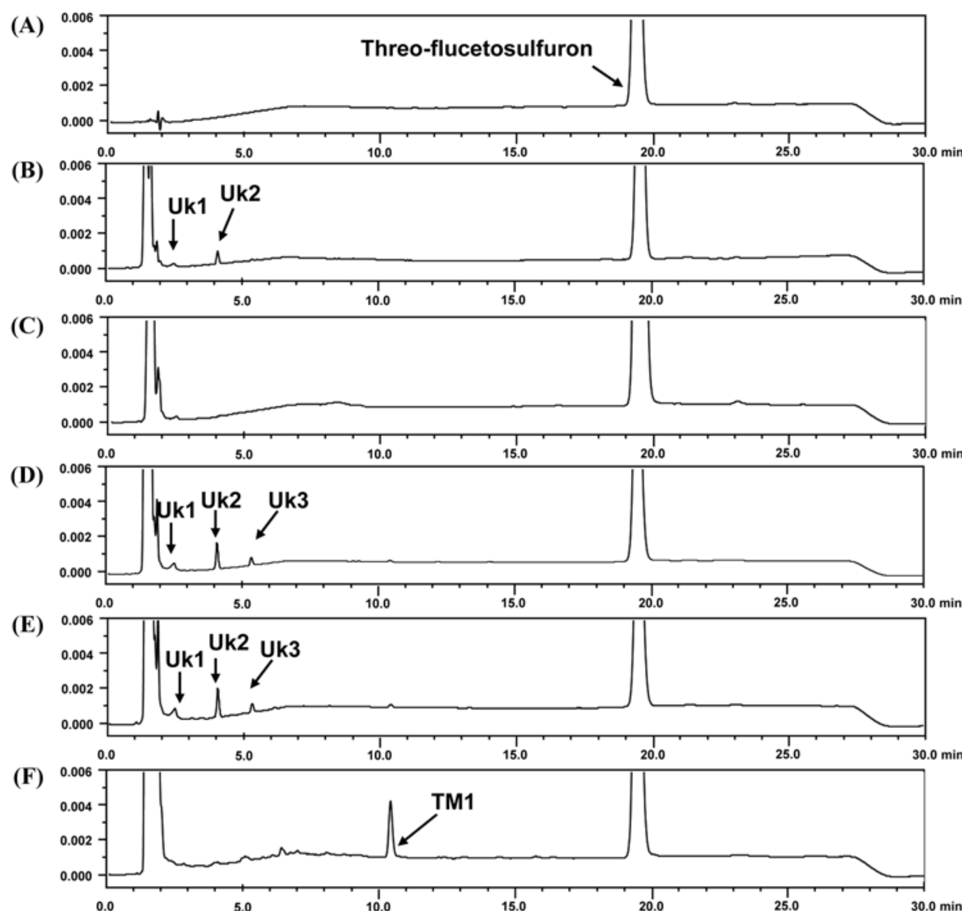

Fig. 2 HPLC profiles of threo-flucetosulfuron in gastrointestinal solutions, buffer solution pH 7.4 (A), buffer solution pH 2 (B), saliva (C), saliva + gastric juice (1:4 v/v) (D), gastric juice (E), and intestinal juice (F).

μM. The stocks diluted at the concentration of calibration curves were fitted with high linearity (r^2 >0.997).

Results and Discussion

Preparation of artificial GI juices. Prior to study on *in vitro* metabolism of flucetosulfuron by GI juices, attempt was made to prepare similar artificial GI juices to those in the human body. Because the human saliva, with pH ranging from 5.3 to 7.8, is 99.5% water and 0.5% other components such as proteins, enzymes, mucins, nitrogenous products, and electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates, the artificial saliva that we prepared had pH 7.4 and contained all of the above components (Young and Schneyer, 1981; Humphrey and Williamson, 2001; Adenugba et al., 2008). To ensure optimum pH (1–3.5) for the denaturation of proteins from food intake and reaction of the enzyme pepsin, the gastric juice was prepared to pH 2 by using HCl, KCl, and NaCl with pepsin and mucin (Gray and Bucher, 1941; Lian et al., 2003; Ganong and Barrett, 2005; Adenugba et al., 2008).

Intestinal juice was mixed with pancreatin, trypsin, bile, and electrolytes. Pancreatin and trypsin are secreted by the pancreas, whereas bile is released by the liver through the bile duct. Other components in intestinal juice are secretions by the intestinal glands in the small intestine (Borgström et al., 1957). Therefore, the intestinal juice was prepared by mixing pancreatin, trypsin, bile, urea, and electrolytes, and the pH was adjusted to 7.4 (Rao et al., 1989; Adenugba et al., 2008).

Studies have shown that the compositions of each GI juice can be diversely modified according to the purpose of the study. For our study on the *in vitro* metabolism of flucetosulfuron, we used the same composition of GI juices used by Adenugba et al. (2004), because in this composition, several major enzymes and salts were present, which is similar to the GI juice content in the human body.

***In vitro* metabolic reaction of flucetosulfuron by artificial GI juices.** To determine the flucetosulfuron metabolism in artificial saliva, saliva + gastric juice (1:4 v/v), gastric juice, and intestinal juice, the GI solutions with 100 μM of each flucetosulfuron isomer were incubated for a specific time. Metabolic reaction times to satisfy the proper digesting time in the body were determined to

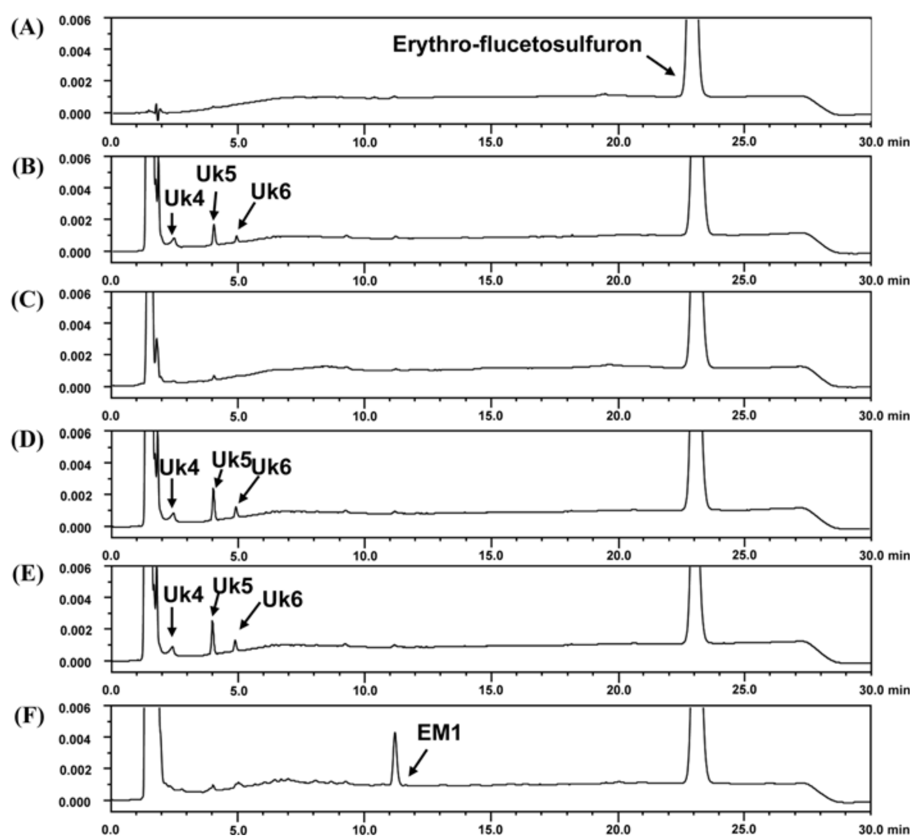

Fig. 3 HPLC profiles of erythro-flucetosulfuron in gastrointestinal solutions, buffer solution pH 7.4 (A), buffer solution pH 2 (B), saliva (C), saliva + gastric juice (1:4 v/v) (D), gastric juice (E), and intestinal juice (F).

5 min for saliva, 2 h for acidic control, saliva + gastric juice (1:4 *v/v*), and gastric juice, and 6 h for neutral control and intestinal juice, by considering the digesting time required by the GI organs such as the mouth, stomach, and small intestine. The metabolite peaks in the HPLC chromatogram of the incubated reaction with 100 M threo-flucetosulfuron were identified for the artificial GI juices (Fig. 2). Threo-flucetosulfuron did not show any metabolite in the control, which was a pH 7.4 buffer solution, whereas Uk2 and Uk3 were observed as unknown metabolites under acidic conditions, namely pH 2. In artificial saliva (5 min), threo-flucetosulfuron was stable. The reaction with artificial saliva + gastric juice (1:4 v/v) and artificial gastric juice for 2 h each produced some metabolites such as Uk1, Uk2, and Uk3, with no interfering peaks. The retention times of Uk1, Uk2, and Uk3 were 2.45, 4.05, and 5.03 min, respectively. TM1 was the only metabolite in the reaction mixture with intestinal juice for 6 h, indicating that flucetosulfuron can be catalyzed by trypsin, pancreatin, or bile, by the breakdown of its ester bond.

Erythro-flucetosulfuron also shows a similar metabolism pattern as that by the threo-isomer (Fig. 3). Reaction mixtures with saliva + gastric juice (1:4 v/v) and artificial gastric juice for 2 h each produced metabolites such as Uk4, Uk5, and Uk6, which eluted at 2.45, 4.05, and 4.95 min, respectively. No other metabolite peaks or interfering peaks were observed in the chromatograms.

Identification of unknown metabolites. To identify the structure of unknown metabolites, the metabolic reaction mixtures were analyzed using LC-MS/MS. From the LC-MS/MS fragmentation patterns, we observed that the metabolites showed the same $[M+H]^+$ at m/z 416 as those after the *in vitro* metabolism of flucetosulfuron by HLMs, indicating that TM1 and EM1 can be formed in the intestinal juice (Fig. 4A). TM1 and EM1 were already identified as the main metabolites of flucetosulfuron isomers in *in vitro* metabolism of flucetosulfuron by HLMs (Lee et al., 2014).

Furthermore, the LC-MS/MS analysis showed that Uk1 and Uk4 in the reaction mixture of gastric juice and saliva + gastric juice (1+4, v/v) had the same $[M+H]^+$ at m/z 235 as those of the fragment ions at *m/z* 106, 136, 200, and 217 (Fig. 4B), indicating cleavage of the pyrimidine moiety from the parent flucetosulfuron isomers ($[M+H]^+$ = m/z 488). The results suggested that the metabolites, Uk1 and Uk4 are hydrolysis product, M2: threo-M2 (TM2) and erythro-M2 (EM2), respectively.

The LC-MS/MS spectra of Uk2 and Uk5 ($[M+H]^+$ = m/z 156) showed fragment ions at *m/z* 112 and 140 (Fig. 4C). Uk2 and Uk5 were identified as M3, which may have been formed by the cleavage of the sulfonylurea bridge.

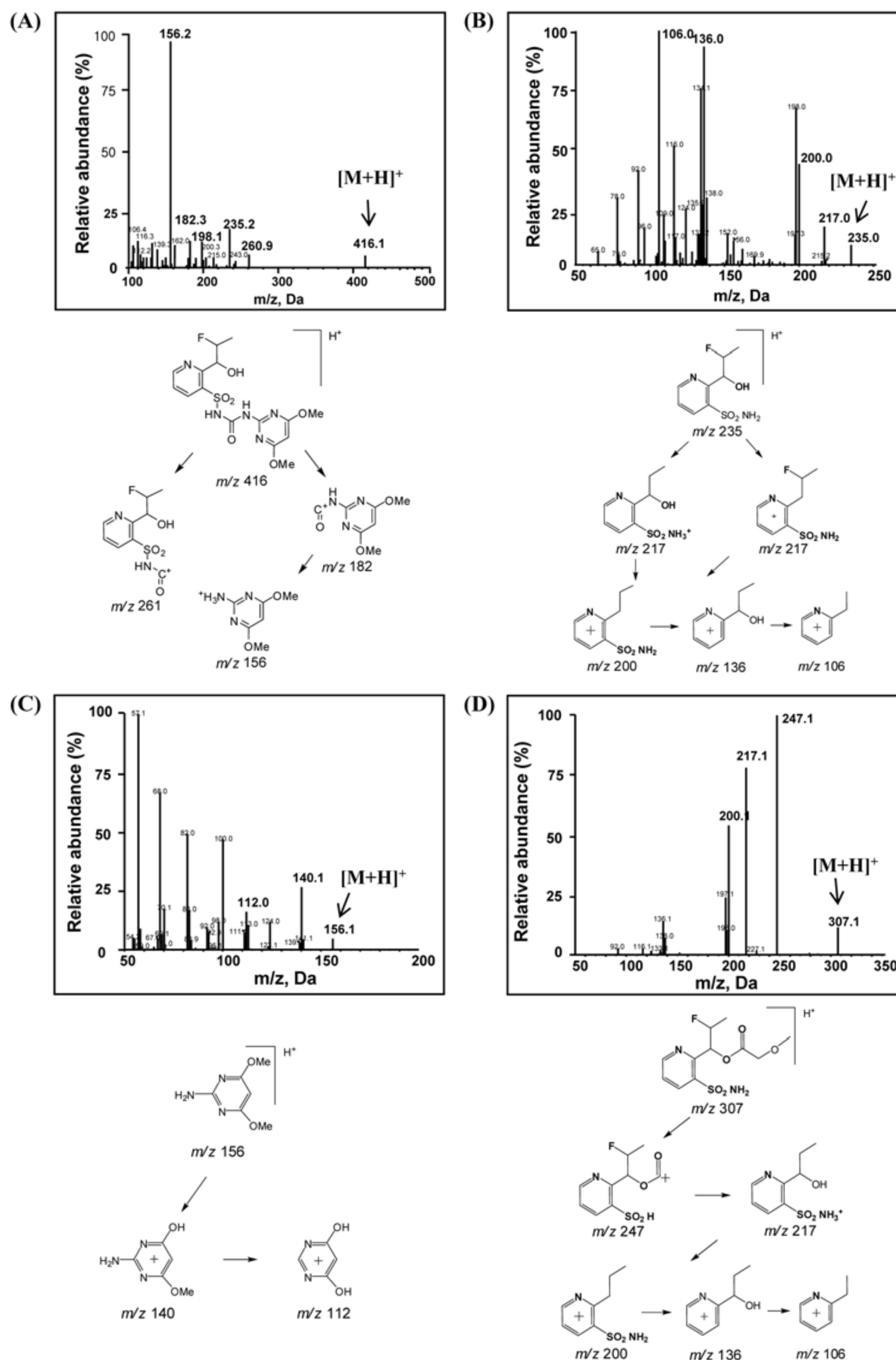

Fig. 4 LC-MS/MS Spectra and fragmentation of metabolites, M1 (A; $[M+H]^+$ = m/z 416), M2 (B; $[M+H]^+$ = m/z 235), M3 (C; $[M+H]^+$ = m/z 156), and M4 (D; $[M+H]^+$ = m/z 307).

The LC-MS/MS spectra of Uk3 and Uk6 ($[M+H]^+$ = m/z 307) showed fragmentation ions at *m/z* 106, 136, 200, 217, and 247 (Fig. 4D), indicating that Uk3 and Uk6 is M4, formed by the cleavage of the sulfonylurea bridge. Uk3 and Uk6 were in the threo (TM4) and erythro (EM4) forms, respectively.

By comparing the LC-MS/MS fragmentation spectra and co-chromatography, the metabolites that formed in the GI juices were found to be identical. Thus, in the case of oral intake of flucetosulfuron, we confirmed that the metabolites of flucetosulfuron in the GI juices were easily hydrolysable at the sulfonylurea bridge

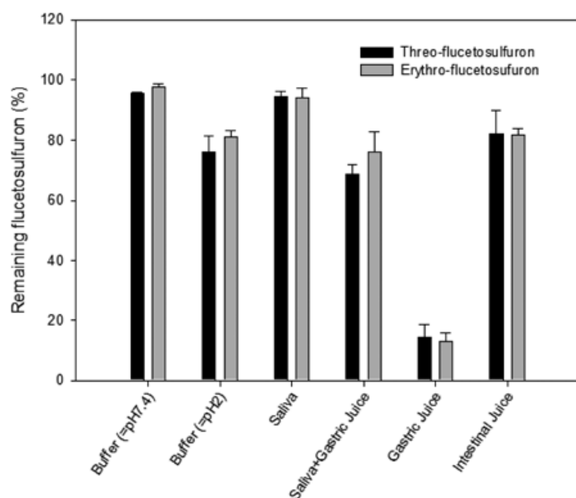

Fig. 5 Remaining flucetosulfuron after metabolic reaction with artificial gastrointestinal juices.

or ester bond, under normal digestion conditions.

The metabolite formation patterns, with only threo-forms—TM1, TM2, and TM4 from threo-flucetosulfuron and only erythro-forms—EM1, EM2, and EM4 from erythro-flucetosulfuron by artificial GI juices, indicated no possibility of chiral conversion between the flucetosulfuron isomers or their metabolites during metabolic reaction with the GI juices.

Metabolic pattern of flucetosulfuron by artificial GI juices. The degradation patterns of flucetosulfuron isomers in each artificial GI juice are shown in Fig. 5. Flucetosulfuron isomers were relatively stable in the buffer solution of pH 7.4 and in artificial saliva, whereas they were easily degraded under acidic condition at pH 2 within 2 h.

In gastric juice, only 14.5% of threo-flucetosulfuron and 13.1% erythro-flucetosulfuron remained, indicating that both the isomers were extremely unstable in artificial gastric juice (Fig. 5). After 2 h incubation in the reaction mixture of artificial saliva + gastric juice (1:4 v/v), flucetosulfuron isomers decreased to 68.9–76.1%, showing that both flucetosulfuron isomers were unstable. The metabolites M2 (TM2 or EM2), M3, and M4 (EM4 or TM4) were produced in the reaction mixture of gastric juice and saliva + gastric juice (1:4 v/v), indicating that acidic pH and pepsin had catalytic effects on the formation of metabolites through breakdown of the sulfonylurea bridge and ester bond. M1 (TM1 or EM1) was also observed under acidic conditions such as saliva + gastric juice (1:4 v/v); however, the level was under the limit of quantification, indicating that the sulfonylurea bridge is the most prone to be hydrolyzed under acidic conditions (Tables 2 and 3).

On the other hand, the residues of flucetosulfuron in the metabolic reaction mixture of artificial intestinal juice were 82.1% for threo-isomer and 81.8% for erythro-isomer, indicating that flucetosulfuron is slightly unstable in artificial intestinal juice. TM1 from threo-flucetosulfuron and EM1 form erythro-flucetosulfuron were the main metabolites in the intestinal juice, with no other metabolites observed. Considering that the pH of the artificial intestinal juice was adjusted to 7.4, a stable condition for flucetosulfuron, it was estimated that trypsin, pancreatin, and bile in the intestinal juice exerted hydrolytic effects on flucetosulfuron. No significant differences were observed in the degradation patterns between flucetosulfuron isomers in each artificial GI juice.

Table 2 Metabolism of threo-flucetosulfuron (100 μM) by artificial gastrointestinal juices

	Gastrointestinal Juices	Optimal time	Concentration (μM)				
			TM1	TM2	M3	TM4	Flucetosulfuron
Threo-flucetosulfuron	Buffer (pH 7.4)	6 h	-	-	-	-	95.7±2.9
	Buffer (pH 2)	2 h	-	0.75±0.13	0.58±0.16	-	76.1±5.3
	Saliva	5 min	-	-	-	-	94.7±1.7
	Saliva + Gastric Juice (1:4 v/v)	2 h	<0.2[a]	2.48±0.12	1.91±0.87	1.71±0.10	68.9±1.8
	Gastric Juice	2 h	<0.2[a]	2.12±0.11	2.86±0.02	1.42±0.26	14.5±2.0
	Intestinal Juice	6 h	4.44±0.59	-	-	-	82.1±7.7

[a]Observed under LOQ.

Table 3 Metabolism of erythro-flucetosulfuron (100 μM) by artificial gastrointestinal juices

	Gastrointestinal Juices	Optimal time	Concentration (μM)				
			EM1	EM2	M3	EM4	Flucetosulfuron
Erythro-flucetosulfuron	Buffer (pH 7.4)	6 h	-	-	-	-	95.2±3.3
	Buffer (pH 2)	2 h	-	0.70±0.18	1.30±0.14	-	81.3±0.2
	Saliva	5 min	-	-	-	-	94.2±3.3
	Saliva + Gastric Juice (1:4 v/v)	2 h	<0.2[a]	2.35±0.71	2.70±0.32	1.17±0.17	76.1±6.9
	Gastric Juice	2 h	<0.2[a]	2.99±0.14	2.82±0.05	1.26±0.30	13.1±2.6
	Intestinal Juice	6 h	5.09±0.13	-	-	-	81.8±2.2

[a]Observed under LOQ.

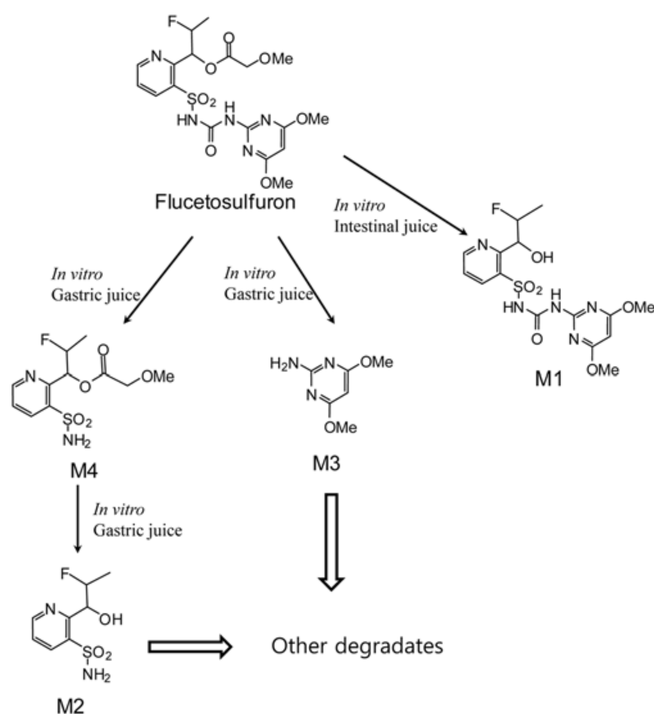

Fig. 6 Proposed metabolic pathway of flucetosulfuron in *in vitro* metabolism by artificial gastrointestinal juices.

Because it is impossible to perform *in vivo* metabolism of pesticides in humans (Versantvoort et al., 2004), the current *in vitro* metabolism of flucetosulfuron was designed to provide an ideal estimation of the *in vivo* situation in humans. In this model, both flucetosulfuron isomers were rapidly degraded within 2 h in the gastric juices, and the degradation rate was higher than 85% of flucetosulfuron.

To date, some researchers have suggested pesticide biomonitoring approach by using animal and human salivas, or artificial GI juices. But, there was limited information on metabolism of pesticides by GI juices (Nigg et al., 1993; Lu et al., 2003; Timchalk et al., 2007; Lee, 2008; Esteban and Castaño, 2009). Our study showed that flucetosulfuron could be degraded in the GI organs, especially in the stomach (~85% degradation) and intestines (~18% degradation), before reaching other metabolic organs such as the liver or cytosol through the blood stream.

On the basis of these results, the proposed metabolic pathway of flucetosulfuron by artificial gastric juices is shown in Fig. 6. The present study showed that there is no significant difference in the degradation patterns between flucetosulfuron isomers in each artificial GI juice. Considering the rapid degradation of flucetosulfuron by artificial GI juices in the *in vitro* metabolism study, the availability of flucetosulfuron from the GI tract into the blood stream is considered limited in the case of accidental oral intake of flucetosulfuron, and hence, the possibility of inducing any serious human toxicity is expected to be negligible.

Acknowledgment The authors thank LG Life Sciences, Korea, for providing flucetosulfuron and metabolites.

References

Abass K, Turpeinen M, Rautio A, Hakkola J, and Pelkonen O (2012) Metabolism of pesticides by human cytochrome P450 enzymes *in vitro* - a survey. In *Insecticides-advances in integrated pest management,* Perveen F (ed.), pp. 165–94. Intech Europe, Croatia.

Abrahamsson B, Albery T, Eriksson A, Gustafsson I, and Sjberg M (2004) Food effects on tablet disintegration. *Europ J Pharmaceu Sci* **22**, 165–72.

Adenugba AA, Mcmartin DW, and Beck AJ (2008) *In vitro* approaches to assess bioavailability and human gastrointestinal mobilization of food-borne polychlorinated biphenyls (PCBs). *J Environ Sci Health* **43**, 410–21.

Borgström B, Dahlqvist A, Lundh G, and Sjövall J (1957) Studies of intestinal digestion and absorption in the human. *J Clin Invest* **36**, 1521.

Chang SS, Lu TH, Eddleston M, Konradsen F, Sterne JA, Lin JJ et al. (2012) Factors associated with the decline in suicide by pesticide poisoning in Taiwan: A time trend analysis, 1987–2010. *Clin Tox* **50**, 471–80.

Esteban M and Castaño A (2009) Non-invasive matrices in human biomonitoring: A review. *Environ Int* **35**, 438–49.

Ganong WF and Barrett KE (2005) Digestion & Absorption. In *Review of medical physiology*. pp. 384–93. McGraw-Hill Medical New York, USA.

Gorbach SL, Barakat S, and Gualtieri L (1992) Survival of *Lactobacillus species* (*strain GG*) in human gastrointestinal tract. *Digest Diseas Sci* **37**, 121–8.

Gray J and Bucher GR (1941) The composition of gastric juice as a function of the rate of secretion. *Americ J Physic-Leg Cont* **133**, 542–50.

Humphrey SP and Williamson RT (2001) A review of saliva: normal composition, flow, and function. *J Prosth Dent* **85**, 162–9.

Hur SJ, Lim BO, Decker EA, and McClements DJ (2011) *In vitro* human digestion models for food applications. *Food Chem* **125**, 1–12.

Jei MS (1992) A Study on the Misuses of Pesticides for Suicides. *Kor Soc Pub Health Nurs* **6**, 62–70.

Lee HK, Moon JK, Chang CH, Choi H, Park HW, Park BS et al. (2006) Stereoselective metabolism of endosulfan by human liver microsomes and human cytochrome P450 isoforms. *Drug Metab Dispos* **34**, 1090–5.

Lee JH (2008) Fate of boscalid on cucumber and its metabolism in *in vitro* biological system. MS Thesis, Seoul National University, Korea.

Lee MG, Shim JH, Ko S, and Chung HR (2010) Research trends on the development of scientific evidence on the domestic maximum residue limits of pesticides. *Food Sci Ind* **43**, 41–66.

Lee S (2010) The occupational diseases of agricultural workers. *Hanyang Med Review* **30**, 305–12.

Lee YS, Liu KH, Moon JK, Ko BJ, Choi H, Hwang KS et al. (2014) *In vitro* metabolism of flucetosulfuron by human liver microsomes. *J Agr Food Chem* **62**, 3057–63.

Lian WC, Hsiao HC, and Chou CC (2003) Viability of microencapsulated bifidobacteria in simulated gastric juice and bile solution. *Int J Food Microbiol* **86**, 293–301.

Lu C, Irish R, and Fenske R (2003) Biological monitoring of diazinon exposure using saliva in an animal model. *J Toxicol Environ Health* **66**, 2315–25.

Nigg HN, Stamper JH, and Mallory L (1993) Quantification of human exposure to ethion using saliva. *Chemosphere* **26**, 897–906.

Rao A, Shiwnarain N, and Maharaj I (1989) Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juices. *Canadian Inst Food Sci Tech J* **22**, 345–9.

Roh S (2012) Work-related diseases of agricultural workers in south korea. *J Kor Med Assoc* **55**, 1063–9.

Tao S, Li L, Ding J, Zhong J, Zhang D, Lu Y et al. (2010) Mobilization of Soil-Bound Residue of Organochlorine Pesticides and Polycyclic

Aromatic Hydrocarbons in an *in vitro* Gastrointestinal Model. *Environ Sci Tech* **45**, 1127–32.

Tao S, Lu Y, Zhang D, Yang Y, Yang Y, Lu X et al. (2009) Assessment of oral bioaccessibility of organochlorine pesticides in soil using an *in vitro* gastrointestinal model. *Environ Sci Tech* **43**, 4524–9.

Timchalk C, Campbell JA, Liu G, Lin Y, and Kousba AA (2007) Development of a non-invasive biomonitoring approach to determine exposure to the organophosphorus insecticide chlorpyrifos in rat saliva. *Toxicol appl Pharm* **219**, 217–25.

Versantvoort CH, Oomen AG, Van de Kamp E, Rompelberg CJ, and Sips AJ (2005) Applicability of an *in vitro* digestion model in assessing the bioaccessibility of mycotoxins from food. *Food Chem Toxico* **43**, 31–40.

Virkel G, Carletti M, Cantiello M, Della Donna L, Gardini G, Girolami F et al. (2009) Characterization of xenobiotic metabolizing enzymes in bovine small intestinal mucosa. *J Vet Pharm Thera* **33**, 295–303.

Yang AG, Shim KH, Choi OJ, Park JH, Do JH, Hwang IG et al. (2012) Establishment of the Korean total diet study (TDS) model in consideration to pesticide intake. *Korean Soc Pest Sci* **16**, 151–62.

Yang SK, Chang WC, and Huang JD (1993) Effects of sodium fluoride and cobalt chloride on the enantioselectivity of microsomal and cytosolic esterases in rat intestinal mucosa. *Biochem Pharm* **46**, 1511–4.

Young J and Schneyer CA (1981) Composition of saliva in mammalia. *Austral J Exp Bio Med Sci* **59**, 1–53.

Zhang J and Li Z (2012) Characteristics of Chinese rural young suicides by pesticides. *Int J Soci Psych* **59**, 655–62.