

Research Article



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주요 농산물 중 Benzenesulfonamide계 살균제 Flusulfamide의 잔류 분석법

안경근¹, 김기쁨¹, 황영선², 강인규³, 이영득⁴, 정명근^{1*}

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Analytical Method for Flusulfamide as Benzenesulfonamide Fungicide, Residues in Major Agricultural Commodities

Kyung-Geun Ahn¹, Gi-Ppeum Kim¹, Young-Sun Hwang², In-Kyu Kang³, Young Deuk Lee⁴ and Myoung-Gun Choung^{1*} (¹Department of Herbal Medicine Resource, Kangwon National University, Samcheok 25949, Korea, ²Department of Biology, University of Texas-Arlington, Arlington, TX 76019, USA, ³Department of Horticultural Science, Kyungpook National University, Daegu 41566, Korea, ⁴Division of Life and Environmental Science, Daegu University, Gyeongsan 38453, Korea)

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ORCID

Myoung-Gun Choung

<http://orcid.org/0000-0002-4391-5513>

Abstract

BACKGROUND: An analytical method was developed using HPLC-UVD/MS to precisely determine the residue of flusulfamide, a benzenesulfonamide fungicide used to inhibit spore germination.

METHODS AND RESULTS: Flusulfamide residue was extracted with acetone from representative samples of five raw products which comprised apple, green pepper, Kimchi cabbage, hulled rice, and soybean. The extract was diluted with large volume of saline water and directly partitioned into dichloromethane to remove polar co-extractives in the aqueous phase. For the hulled rice and soybean samples, *n*-hexane/acetonitrile partition was additionally employed to remove non-polar lipids. The extract was finally purified by optimized Florisil column chromatography. On an octadecylsilyl column in HPLC, flusulfamide was successfully separated from co-extractives of sample, and sensitively quantitated by ultraviolet absorption at 280 nm

with no interference. Accuracy and precision of the proposed method was validated by the recovery experiment on every crop sample fortified with flusulfamide at 3 concentration levels per crop in each triplication.

CONCLUSION: Mean recoveries ranged from 82.3 to 98.2% in five representative agricultural commodities. The coefficients of variation were all less than 10%, irrespective of sample types and fortification levels. Limit of quantitation (LOQ) of flusulfamide was 0.02 mg/kg as verified by the recovery experiment. A confirmatory method using LC/MS with selected-ion monitoring technique was also provided to clearly identify the suspected residue.

Key words: Flusulfamide, HPLC-UVD/MS, Residue

서론

Flusulfamide (2',4-dichloro- α,α,α -trifluoro-4'-nitro-m-toluenesulfonanilide; Fig. 1) 1992
benzenesulfonamide (Kramer *et al.*, 2011),

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(Kidd and

*Corresponding author: Myoung-Gun Choung
Phone: +82-33-540-3321; Fax: +82-33-540-3329;
E-mail: cmg7004@kangwon.ac.kr

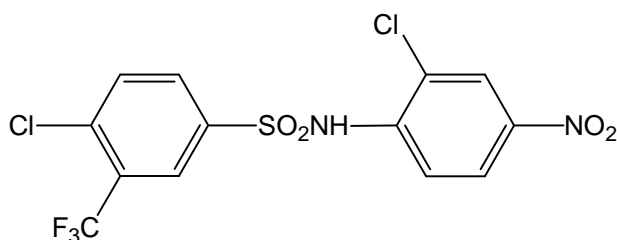


Fig. 1. Chemical structure of flusulfamide.

David, 1991; Tanaka *et al.*, 1999).

(Korea Crop Protection Association, 2015),
(Maximum Residue
Limits; MRL) 0.05 mg/kg (Ministry
of Food and Drug Safety, 2014).

Flusulfamide *n*-octanol/
water (Log Pow) 2.8
170-172.5°C, 415.17 (C₁₃H₇C₁₂F₃N₂O₄S)
(Kramer *et al.*, 2011).

(acetone 314.0 g, chloroform 17.0 g,
ethyl acetate 125.0 g, methanol 24.0 g, tetrahydrofuran
592.0 g/L), 2.9 mg/L
(Kramer *et al.*, 2011).

Benzenesulfonamide flusulfamide
GC/MS (Gas chromatograph-mass spectrometry)
GC-ECD/MS (Gas chromatograph-electron capture
detector/mass spectrometry)
(Chou *et al.*, 2004; Park *et al.*, 2009),
SPE (Solid phase extraction)

가, HPLC-
UVD (High performance liquid chromatograph-ultra
violet detector) LC-MS (Liquid chromatograph-
mass spectrometry) (Yoshii
et al., 2000; Kitayama *et al.*, 2002),
가, LC-MS

benzenesulfonamide
flusulfamide 가,

재료 및 방법

시약 및 기구

flusulfamide Dr.
Ehrenstorfer GmbH (Germany) 97.0%
methanol
1,000 mg/L 가 stock solution
-20°C acetonitrile
J. T. Baker (USA) Florisil
(60-100 mesh) 130°C 가
J. T. Baker (USA)
HPLC, acetone, *n*-hexane,
dichloromethane, methanol acetonitrile
Eyela NE-1000SW (Japan)
(IKA, Ultra-
Turrax T-25, USA)

농산물 시료

(Ministry of Food and Drug
Safety, 2014) flusulfamide
Codex
(Codex, 2003)
(Lee *et al.*, 2010).
(Ministry of
Food and Drug Safety, 2012)

HPLC-UVD/MS 기기분석 조건

Flusulfamide sulfonamide
가

Table 1. HPLC-UVD operating parameters for the analysis of flusulfamide

Instrument	Agilent 1200 HPLC system
Detector	Ultra violet detector (UVD)
Column	YMC-Pack Pro C ₁₈ RS (4.6×250 mm, 5 μm)
Column temp.	40°C
Mobile phase	Acetonitrile/0.5% formic acid-water (80/20, v/v)
Flow rate	0.8 mL/min
Wavelength	UV 280 nm
Sample size	20 μL

Table 2. LC/MS operating parameters for the confirmation of flusulfamide

Instrument	Agilent 6110 Quadrupole LC/MS
Column	YMC-Pack Pro C ₁₈ RS (2.0×150 mm, 3 μm)
Column temp.	40°C
Mobile phase	Acetonitrile/0.5% formic acid-water (80/20, v/v)
Flow rate	0.8 mL/min
Sample size	10 μL
Ionization	Electrospray ionization (ESI), negative-ion mode
Drying gas	N ₂ , 10.0 L/min
Gas temp.	350°C
Capillary voltage	3.0 kV
Mass range (<i>m/z</i>)	200~600

GLC (Gas-Liquid Chromatography)	acetone 40 mL
HPLC conjugation system	1 L
sulfonamide	50 mL
(ultra	450 mL
-	가
dichloromethane	<i>n</i> -hexane, 2
dichloromethane	<i>n</i> -hexane/ 4
Agilent (USA) 1200 series	-
column YMC-Pack Pro C ₁₈ RS (4.6×250 mm, 5 μm)	-
LC/MS (Liquid	가
Chromatograph/Mass Spectrometry)	dichloromethane 50
Agilent	dichloromethane
(USA) 6110 Quadrupole LC/MS	40°C
Table 1 Table 2	-
표준검량선 및 분석정량한계(Limit of quantitation, LOQ)	<i>n</i> -hexane/dichloromethane (90/10, v/v) 10 mL
Flusulfamide stock solution	Florisil
0.05-10 mg/L	가
가 working solution	acetonitrile
20 μL	<i>n</i> -hexane 40 mL
HPLC peak	250 mL
(reproducibility)	<i>n</i> -hexane
acetonitrile 40 mL	2
acetonitrile 40°C	-
0.5 mg/L flusulfamide	<i>n</i> -hexane/dichloromethane (90/10, v/v) 10 mL
HPLC 15 retention	Florisil
time (Rt.) peak area	가
-	-
(Ahn <i>et al.</i> , 2014),	Florisil 흡착 크로마토그래피
flusulfamide	가
-	-
(mg/kg)=[Florisil
(ng)/ (μL)] ×	40×1.5 cm
[(mL)/ (g)]	가
acetone	Florisil
시료의 추출 및 분배	가
25 g acetone 100 mL	<i>n</i> -hexane 50 mL
가 (<i>n</i> -hexane/dichloromethane (90/10,
30 20 mL	v/v) 10 mL
가	가
2	3 mL/min
(12,000 rpm)	-
(Toyo No.	<i>n</i> -hexane/acetone (75/25, v/v) 100 mL
6, Japan) Büchner funnel	가
-	<i>n</i> -hexane/acetone (60/40, v/v) 150

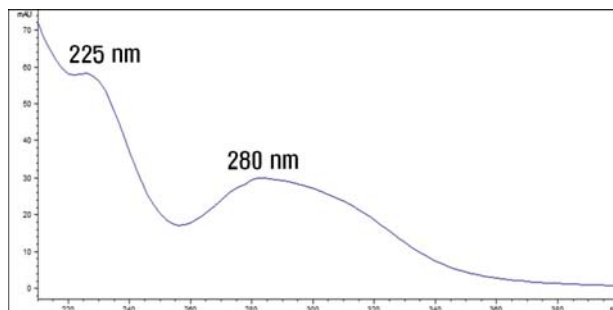
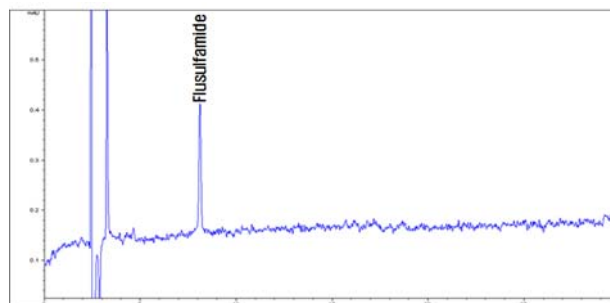


Fig. 2. UV absorption spectrum of flusulfamide.

Fig. 3. Chromatogram of flusulfamide standard solution (20 μ L of 0.05 mg/L in acetonitrile).

mL flusulfamide
Flusulfamide가 40°C
acetonitrile 10 mL HPLC

Flusulfamide의 회수율 검증
flusulfamide

25 g
10 50
flusulfamide 3

Pro C₁₈ RS (4.6×250 mm, 5 μ m)
acetonitrile peak
, acetonitrile/0.5% formic acid-
water (80/20, v/v) (isocratic)
가
(Fig. 3), flusulfamide 8.3
(gradient elution)
, isocratic
가

결과 및 고찰

HPLC 분석조건의 확립

Flusulfamide HPLC
acetonitrile 5 mg/L on-line
HPLC/DAD 190-400 nm
(λ max) 225 nm 280 nm
(Fig. 2), flusulfamide
225 nm 가가
(UV cut-off)

isocratic
HPLC
(Table 1) flusulfamide
S/N (S/N \geq 10) 1
ng , 가
0.5 mg/L 15
(retention time) peak (peak area)
(CV, %) 가
0.88%
가

(Table 3).
가 flusulfamide (0.05-
225 nm 280 nm 10 mg/L) 20 μ L HPLC ,
y=38.4885x-0.1730 (R^2 =0.999**) (Fig. 4). , flusulfamide

HPLC column C₁₈ YMC-Pack

Table 3. Reproducibility of peak area and retention time of flusulfamide using HPLC-UV

Parameter	Retention time (min)	Peak Area (mAU)
Minimum	8.235	19.8
Maximum	8.392	20.3
Mean	8.307	20.1
SD	0.05	0.18
CV (%)	0.59	0.88

*Abbreviations : SD, standard deviation; CV, coefficient of variance.

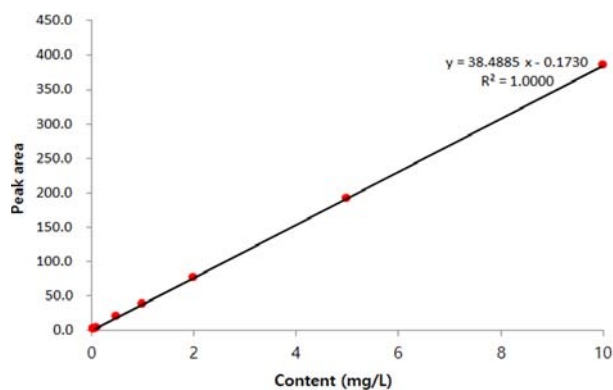


Fig. 4. Calibration curve of flusulfamide in HPLC.

1 ng 200 200 ng
가 $R^2=0.999^{**}$

시료 추출 및 분배과정의 확립
flusulfamide acetone
. Acetone

가

1
가

FDA AOAC -
(AOAC, 2000; Lee *et al.*, 2008).
- *n*-hexane, 2 *n*-hexane/

dichloromethane dichloromethane 4
(Lee *et al.*, 2011),
(Table 4). -
, 100 mL *n*-hexane
flusulfamide 33.2%
100 mL *n*-hexane/dichloromethane (80/20, v/v)
88.7%, 100 mL *n*-hexane/
dichloromethane (20/80, v/v) 95.9%,
dichloromethane 50 mL 2
98.1%

가 dichloromethane 50 mL
2 (IV) flusulfamide

Dichloromethane -

dichloromethane
column
(baseline shift and drift)

HPLC

1-3% 20%

가 (US
FDA, 1999; AOAC, 2000). Table 5
n-hexane acetonitrile 2
flusulfamide가 98.8% 가
n-hexane/
acetonitrile I
0.1-0.4%
가

Table 4. Efficiency of liquid-liquid partition of crude extract by different solvents for flusulfamide

Compound	Recovery (%) ¹⁾			
	Partition I ²⁾	Partition II	Partition III	Partition IV
Flusulfamide	33.2 ± 3.0	88.7 ± 1.1	95.9 ± 0.2	98.1 ± 0.1

¹⁾ Mean values of triplicate samples.

²⁾ Partition mixture : 150 mL acetone + 50 mL saturated NaCl + 450 mL distilled water.

I, 100 mL *n*-hexane, II, 100 mL *n*-hexane/dichloromethane (80/20, v/v), III, 100 mL *n*-hexane/dichloromethane (20/80, v/v), IV, 50 mL dichloromethane (×2 times).

Table 5. Efficiency of *n*-hexane/acetonitrile partition for flusulfamide

Compound	Recovery (%) ¹⁾	
	Partition I ²⁾	Partition II
Flusulfamide	98.9±0.5	99.6±0.4

¹⁾ Mean values of triplicate samples.

²⁾ Partition mixture : 40 mL *n*-hexane saturated with acetonitrile.

I, 40 mL acetonitrile saturated with *n*-hexane (×2 times), II, 40 mL acetonitrile saturated with *n*-hexane (×3 times).

Table 6. Elution profile of flusulfamide on Florisil column chromatography

Elution solvent (v/v)	Recovery (%) ¹⁾			
	0-50 mL	51-100 mL	101-150 mL	Total
75 : 25 ²⁾	0.0	0.0	0.0	0.0
70 : 30 ³⁾	1.4	4.4	7.0	12.8
65 : 35 ³⁾	9.3	29.0	43.0	81.3
60 : 40 ³⁾	39.2	53.2	2.6	95.0

¹⁾ 10 g of activated Florisil (60-100 mesh) was packed with solvent.
²⁾ *n*-Hexane/acetone (v/v).
³⁾ Pre-washed with 100 mL of ²⁾ solvent system, and then eluted *n*-hexane/acetone (v/v).

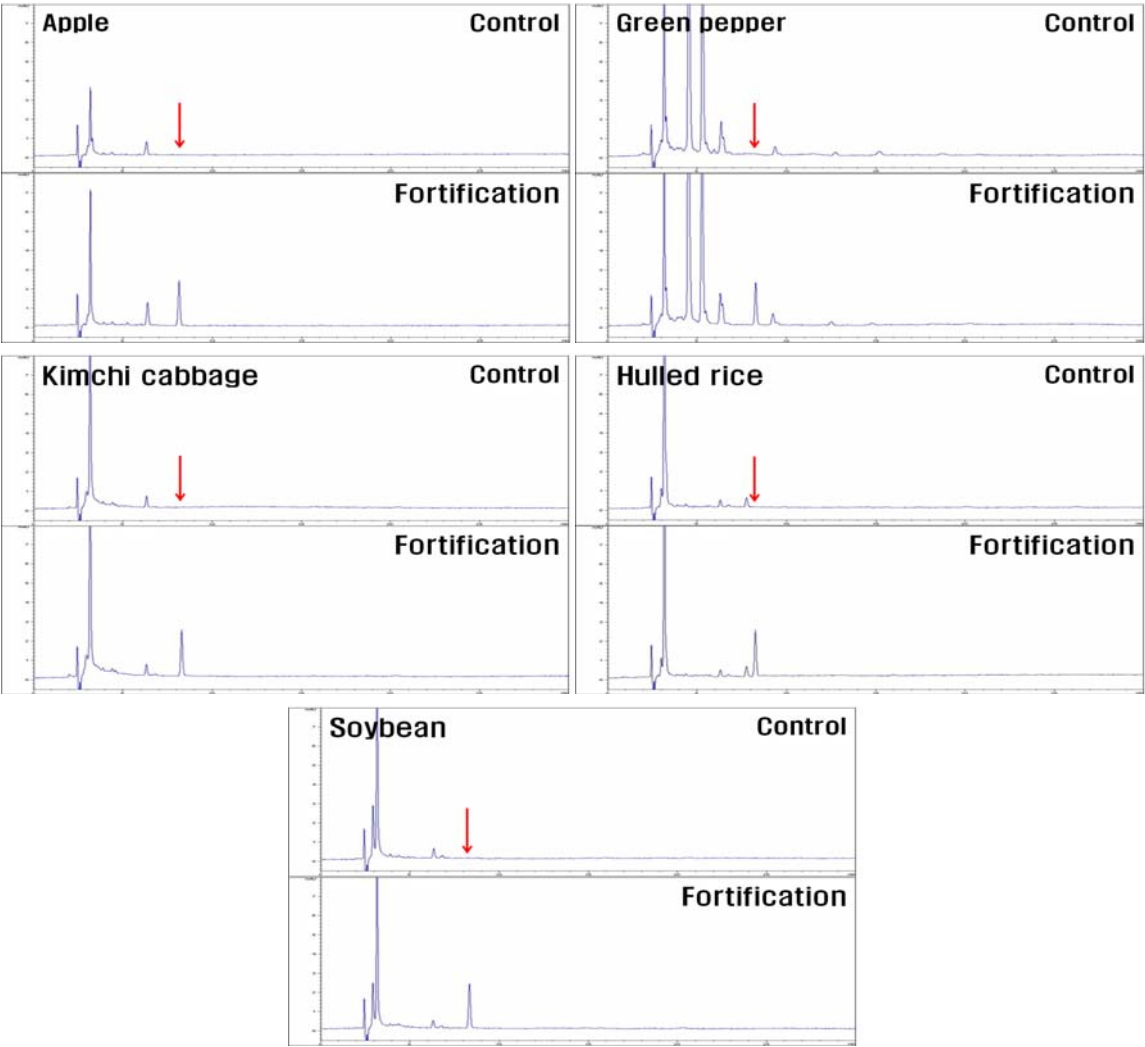


Fig. 5. HPLC chromatograms of typical agricultural commodity extracts for the analysis of flusulfamide.

n-hexane/acetonitrile - , 가
Florisil 흡착 크로마토그래피 정제조건이 최적화 가
flusulfamide - Florisil ,
n-hexane/acetonitrile *n*-hexane/acetone
(Table 6). Florisil

Table 7. Recoveries of flusulfamide with different crop samples

Crop	Fortification (mg/kg)	Recovery (%) ¹⁾	CV (%)	LOQ (mg/kg)
Apple	0.02	91.4±3.0	3.3	0.02
	0.2	91.2±1.0	1.1	
	1	91.1±0.9	0.9	
Kimchi cabbage	0.02	90.5±4.8	5.3	0.02
	0.2	93.0±1.0	1.1	
	1	92.7±0.8	0.8	
Green pepper	0.02	82.3±0.0	0.0	0.02
	0.2	88.4±1.8	2.0	
	1	93.5±0.9	1.0	
Hulled rice	0.02	91.4±6.0	6.5	0.02
	0.2	93.9±2.7	2.8	
	1	95.3±1.4	1.4	
Soybean	0.02	98.2±6.1	6.2	0.02
	0.2	89.2±0.8	0.9	
	1	92.6±1.5	1.6	

¹⁾ Mean values of triplicate samples with standard deviations.

flusulfamide
, *n*-hexane/acetone (75/25, v/v) 100 mL
pre-washing , *n*-hexane/acetone (60/40, v/v) 150 mL
flusulfamide 95.0%
가
Florisil
가
농산물 시료 중 flusulfamide의 분석 정량한계 및 회수율
Fig. 5

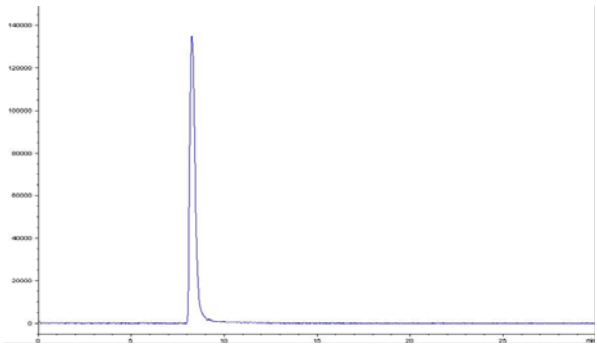


Fig. 6. Total-ion chromatogram (TIC) of flusulfamide in LC/MS.

(LOQ)
flusulfamide 0.02
mg/kg , Codex(Codex Alimentarius
Commission, 2003)
(Lee, 2017)
mg/kg 1/2
50 flusulfamide 가 , 10
3
82.3-98.2%,
10 88.4-93.9%, 50
91.1-95.3% ,
6.5% .
70-120% 10%
(Table 7).
flusulfamide 가
LC/MS를 이용한 잔류분의 재확인
flusulfamide
LC/MS 가
fragment ion 가 (Kwon et
al., 2008).
Flusulfamide HPLC/UVD

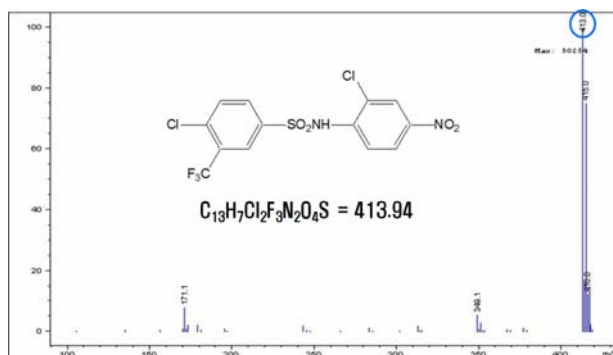


Fig. 7. ESI (-) mass spectrum of flusulfamide.

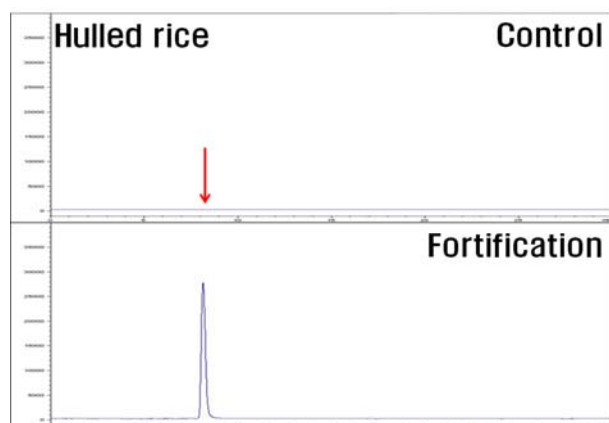


Fig. 8. SIM chromatogram of hulled rice extract for the confirmation of flusulfamide.

acetonitrile/0.5% formic acid-water
formic acid가 LC/MS
가 가
, HPLC/UV
LC/MS
. Fig. 6 7 TIC (total-ion chromatogram)
mass spectrum flusulfamide ESI (electrospray
ionization) negative ion $[M-H]^-$
 m/z 413.0
flusulfamide $[M-H]^-$ peak가 base peak
selected-ion monitoring (SIM) ion $[M-H]^-$
 m/z 413.0 ion 가
(McLafferty and Turecek, 1993; Ardrey, 2003).
Fig. 8
flusulfamide SIM (selected-ion monitoring)
chromatogram ,
, 가
flusulfamide
LC/MS SIM HPLC/
UVD flusulfamide
가 .

요 약

HPLC-UVD/MS
benzenesulfonamide flusulfamide
, acetone flusulfamide
dichloromethane - Florisil
HPLC-UVD/MS
. Flusulfamide
HPLC-UVD , (LOQ)
0.02 mg/kg ,
10 50 ,
82.3-98.2% ,
(CV) 6.5%
70-120% 10%
, LC/MS/SIM
flusulfamide HPLC-UVD/MS ,
가 .

Note

The authors declare no conflict of interest.

Acknowledgement

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