UC Biodevices

Immunoaffinity Column for Confirmatory Test of Sulfonamides (IAC-SULF)

(Product Number: 5501C305)

（Sulfonamides may be carcinogenic）

INSTRUCTION MANUAL

(v. 1.00)

1. Introduction

Sulfonamides (SULF) are widely used as feed additives, mainly for fattening of calves and pigs. Combined with inhibitors of dihydrofolate reductase such as trimethoprim, tetroxoprim, pyrimethamine. Sulfonamides are also used in veterinary medicine for the treatment of intestinal infections, mastitis, pulmonitis and other (systemic) diseases. Therefore sulfonamide residues may occur in food of animal origin such as meat, honey, and milk and were found frequently abroad. According to the related laws and regulations in EU, USA, JAPAN and CHINA, a maximum residue limit for all substances of the sulfonamide-group of 100 ppb in muscle, fat, liver and kidney and of 100 ppb in milk and honey is valid. Most of sulfonamides are required below 50ppb in these commodities.

2. INTENDED USE

A simple and efficient extraction and purification procedure for sulfonamides was developed by means of the immunoaffinity column (IAC-SULF) as a cleanup tool. Sulfonamides content in feeds, honey, aquatic products and animal derived foods are cleaned up by IAC and determined by HPLC or LC-MS. It is a fast, simple, safe and highly accurate method for quantitatively measuring SAs (16 analogs)

16 kinds of Sulfonamides

Sulfacetamide，SA	Sulfisomindine, SIM2
Sulfadiazine，SDZ	Sulfathiazole，ST
Sulfapyridine，SPD	Sulfamerazine，SMR
Sulfamethoxydiazine，SMD	Sulfamethizole，SMTZ
Sulfamethazine，SM2	Sulfachloropyridazine，SCP
Sulfamethoxazole，SMZ	Sulfamonomethoxine，SMM
Sulfisoxazole，SIZ	Sulfachloropyrazine，SPZ
Sulfadimethoxine，SDM	Sulfaquinoxaline，SQX

3. PRINCIPLE

Samples are prepared by mixing with an extraction solution, blending and filtering. The extract is then applied to the sulfonamides immunoaffinity column bound with specific antibodies to Sulfonamides. At this stage, the sulfonamides bind to the antibody on the column. The column is then washed with water to rid the immunoaffinity column of impurities. By passing methanol through the column, the sulfonamides are removed from the antibody. This methanol solution can then be injected into HPLC or LC-MS system.

4. PREPARATION OF SOLUTIONS

4.1 0.1M HCl：Take 8.5mL 38% concentrated hydrochloric acid to dissolve in 1000mL DI water

4.2 pH7.4 PBS：

8.0 g NaCl
2.9 g Na_2HPO_4. $12H_2O$
0.24g KH_2PO_4
0.2 g KCl
dissolved in approximately 990 mL DI water and diluted to 1 liter with DI water

4.3 acetic acid /0.01M PBS (11:989,v/v)：

3.58 g Na_2HPO_4. $12H_2O$
1.56g NaH_2PO_4
11mL acetic acid
dissolved in approximately 980 mL DI water and diluted to 1 liter with DI water

5. METHOD: IAC-SULF Test procedure for Aquatic Products and Animal Derived Food （0-200ppb）

5.1 Sample Extraction

5.1.1 Place 5.0 g of muscle tissue homogenate into 50mL polypropylene centrifuge tube

5.1.2 Add to tube 20 ml ethanol/water (80:20, v/v).

5.1.3 Vortex high speed for 2 minutes, and then shaken for 30min with an orbital shaker

5.1.4 After centrifugation at 3000RPM for 5min, the supernatant was decanted into a clean tube

5.2 Extract Dilution and Filtration

5.2.1 Pipette 5 ml supernatant extract into a clean vessel.
5.2.2 Dilute extract with 45ml distilled water. Mix well.
5.2.3 Filter diluted extract through glass microfiber filter and collect filtrate in a clean container.

5.3 Affinity Chromatography:
5.3.1 Remove two end caps from IAC
5.3.2 Pass 20 ml of filtered extract (10 ml = 0.5g sample equivalent) through the column at a steady slow flow rate of about 1 drop per second.
5.3.3 After extract has completely passed through column, pass 10 ml distilled water through column at about 1-2 drops per second flow rate.
5.3.4 Elute SULF column at flow rate of 1 drop per second with 2.0 ml HPLC grade methanol and collect in a clean glass cuvette.
5.3.5 Dry down eluate under a nitrogen stream at 50°C. Reconstitute with 500µL mobile phase.
5.3.6 Inject into HPLC or LC-MS

5.4 Limit of detection (HPLC):20ppb

6. METHOD: IAC-SULF Test procedure for Feeds (0-500ppb)

6.1 Sample Extraction
6.1.1 Place 10.0 g of ground sample into 50mL polypropylene centrifuge tube
6.1.2 Add to 50 ml methanol/0.1M HCl(70/30,v/v)
6.1.3 Cover tube and shaken for 30min with an orbital shaker
6.1.4 After centrifugation at 3000RPM for 5min, the supernatant was decanted into a clean tube

6.2 Extract Dilution and Filtration
6.2.1 Pipette 1.0 ml supernatant extract into a clean vessel.
6.2.2 Dilute extract with 19 ml pH7.4 PBS Mix well.
6.2.3 Filter diluted extract through glass microfiber filter and collect filtrate in a clean container.

6.3 Affinity Chromatography:
6.3.1 Remove two end caps from IAC
6.3.2 Pass 10 ml of filtered extract (10 ml = 0.1g sample equivalent) through the column at a steady slow flow rate of about 1 drop per second.
6.3.3 After extract has completely passed through column, pass 10 ml distilled water through column at about 1-2 drops per second flow rate.
6.3.4 Elute SULF column at flow rate of 1 drop per second with 2.0 ml HPLC grade methanol and collect in a clean glass cuvette.

6.3.5 Dry down eluate under an Nitrogen stream at 50℃. Reconstitute with 500μL mobile phase.

6.3.6 Inject into HPLC or LC-MS

6.4 Limit of detection (HPLC):100 ppb

7. HPLC Setup:

7.1 Column: Cloversil-C18,4.6×250mm（5um）

7.2 Flow rate: 1.0 mL/min.

7.3 Lamp: deuterium or mercury lamp

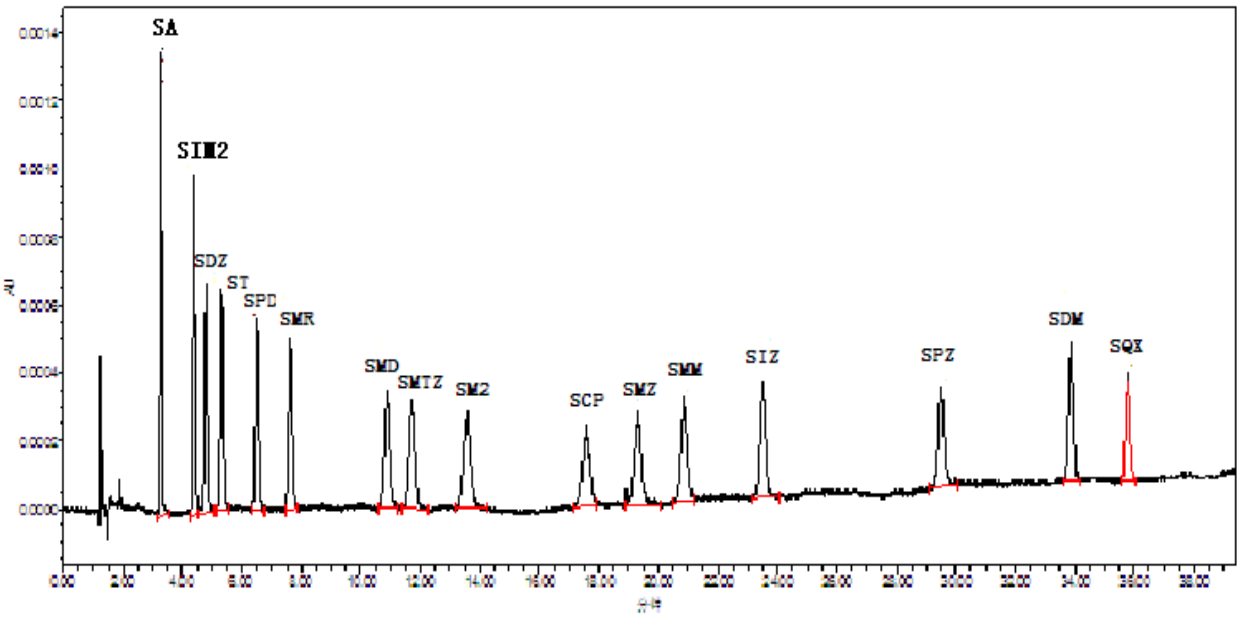
7.4 Detection: 270 nm

7.5 Sample loop: 50-200 μL

7.6 Mobile Phase: Solution A = Methanol

Solution B = acetic acid /0.01M PBS (11:989, v/v)

Time	Mobile Phase A	Mobile Phase B
0	15%	85%
15	15%	85%
40	40%	60%
40.1	15%	85%



HPLC chromatogram of SAs(16 analogs) standard 100ng/mL

8. NOTES

8.1 Storage: IAC-SULF and kits should be stored at 2-8℃. Do not freeze.

8.2 Shelf Life: IAC-SULF columns and kits are stable for 18 months from date of manufacture if stored at 2-8℃.

9. CONTACT INFORMATION

UC BIODEVICES CORP.
3652 Edison Way
Fremont, CA 94538
USA
Tel: 1-510-730-2598
Fax: 1-510-795-1795
www.ucbiodevices.com

August, 2011

50010066 V1.00