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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 29694-2013

**Determination of Sulfonamides residues in animal derived food
by High Performance Liquid Chromatographic method**

食品安全国家标准 动物性食品中13种磺胺类药物多残留的测定 高效液相色谱法
(English Translation)

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1 Scope

This standard specifies the sample preparation and high performance liquid chromatographic method in animal food sulfonamides of Residues.

Sulfacetamide muscle and liver tissue This standard applies to the pigs and chickens, sulfapyridine, sulfanilamide oxazole, sulfamerazine, sulfadiazine Methyl pyrimidine, pyridazine sulfamonomethoxine, sulfanilamide benzoyl, sulfamonomethoxine, sulfanilamide chloropyridazine, sulfamethoxazole, sulfisoxazole, sulfamethazine Pyridazine and oxygen detection pyrazole sulfonamides single or a plurality of drug residues.

2 Normative references

The following documents for the application of this document is essential. For dated references, only applies to the version dated paper Pieces. For undated references, the latest edition (including any amendments) applies to this document.

GB/T 6682 Water for analytical laboratory specifications and test methods Principle 3 Sulfonamides residues in a sample, and extracted with ethyl acetate, 0.1mol/L hydrochloric acid solution was solvent switched, hexane degreasing, MCX column Purification by high performance liquid chromatography - UV method, external standard.

4 Reagents and materials

The following reagents used, unless otherwise stated are analytical reagents, water as a water line with GB/T 6682 provisions.

4.1

sulfacetamide, sulfapyridine, pyridazine sulfamonomethoxine, sulfanilamide benzoyl, sulfamonomethoxine, sulfanilamide chloropyridazine, sulfamethoxazole, sulfonamide iso Oxazole, sulfonamides dimethoxy pyridazine, pyrazole sulfonamides reference. content $\geq 99\%$; sulfa yl, sulfamerazine, sulfamethazine. Content $\geq 98\%$.

4.2

ethyl acetate. chromatography.

4.3

Acetonitrile. chromatographically pure.

4.4

Methanol. HPLC grade.

4.5

hydrochloric acid.

4.6

n-hexane.

4.7

formic acid. HPLC grade.

4.8

ammonia.

4.9

MCX Column. 60mg/3mL, or equivalent person.

4.10 0.1% formic acid solution. Take acid 1mL, dissolved and diluted with water to 1000mL.

4.11 0.1% formic acid in acetonitrile. 0.1% formic acid take 830mL, dissolved in acetonitrile and diluted to 1000mL.

4.12

Eluent. aqueous ammonia take 5mL, dissolved in methanol and diluted to 100mL.

4.13 0.1mol/L hydrochloric acid solution. Take 0.83mL of hydrochloric acid, dissolve and dilute to 100mL with water.

4.14 50% methanol in acetonitrile. Methanol 50mL, dissolved in acetonitrile and diluted to 100mL.

4.15 100µg/mL sulfonamides mixed standard stock solution. Weigh accurately sulfonamides respective standards 10mg, to a 100mL flask, Washed with acetonitrile, dissolved and diluted to the mark, formulated at a concentration of 100µg/mL sulfonamides mixed standard stock solution. -20 °C less, Valid for six months.

4.16 10µg/mL sulfonamides working standard solutions. precise amount of 100µg/mL sulfonamides mixed standard stock solution 5.0 mL, in 50mL volumetric flask, dilute to the mark with acetonitrile, formulated at a concentration of 10µg/mL sulfonamides working standard liquid. -20 °C below, valid for six months.

5 Apparatus

5.1

HPLC. with UV detector or diode array detector.

5.2

Analytical balance. a sense of volume 0.00001g.

5.3

Balance. a sense of the amount of 0.01g.

5.4

vortex meter.

5.5

centrifuge.

5.6

homogenizer.

5.7

rotary evaporator.

5.8

Nitrogen blowing instrument.

5.9

SPE.

5.10

heart-shaped bottle. 100mL.

5.11

PTFE tube. 50mL.

5.12

Membrane. organic phase, 0.22 μ m.

Preparation and Storage of sample 6

6.1 Preparation of the sample

Fresh or thawed take appropriate blank or test tissue, minced, and homogenized.

--- the test sample taken after homogenization, as the feed try.

--- blank sample taken after homogenization, as a blank sample.

--- blank sample taken after homogenization, adding a suitable concentration of the standard working solution, is added as a blank sample.

Save 6.2 sample

Or less at -20 °C.

Determination Step 7

7.1 Extract

Sample Weigh 5g $\pm$ 0.05g, polytetrafluoroethylene in 50mL centrifuge tubes, ethyl acetate was added 20mL, whirl 2min, 4000r/min Centrifuge 5min, supernatant to 100mL heart-shaped flask, the residue was added ethyl acetate 20mL, extraction was repeated once, the two extracts were combined.

7.2 Purification

Heart-shaped flask was added 0.1mol/L hydrochloric acid solution 4mL, concentrated by rotary evaporation to at least 40 °C 3mL, transferred to 10mL centrifuge tubes in. With 0.1mol/L hydrochloric acid solution was washed 2mL heart-shaped bottle, go in the same centrifuge tube. Heart-shaped flask and then washed 3mL hexane, n-hexyl Go alkoxy same centrifuge tube, vortexed 30s, 3000r/min centrifugal 5min, discarded hexane. Again washed with n-hexane heart-shaped 3mL Bottles, full turn in the same centrifuge tube, vortexed 30s, 3000r/min centrifugal 5min, discarded hexane, and the lower layer was reserved.

MCX column washed with methanol and 2mL 0.1mol/L hydrochloric acid solution 2mL activated, taking stock solution through the column, controlling the flow rate 1mL/min.

Sequentially with 0.1mol/L hydrochloric acid solution and 50% methanol 1mL 2mL acetonitrile, and eluted with 4 mL eluate, the eluate was collected, 40 °C dry nitrogen, 0.1% formic acid in acetonitrile was added to dissolve the residue 1.0mL, membrane filtration,