Determination of carrageenan and konjac glucomannan in livestock meat and aquatic products by liquid chromatography-tandem mass spectrometry

1 Scope

The standard specifies the sample preparation and determination of carrageenan and konjac glucomannan by LC-MS/MS in livestock meat and aquatic products.

The standard is applicable to the determination and confirmation of carrageenan and konjac glucomannan in livestock meat and aquatic products.

2 Principle

The carrageenan in the test sample is extracted by homogenizer and hydrolyzed as characteristic oligosaccharide by nitric acid. The final solution is determination by liquid chromatography- triple quadrupole mass spectrometry, quantified by external standard method.

3 Reagents and materials

Unless otherwise specified, all the reagent used should be analytical grade, water is the first grade water prescribed by GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile (CH_3CN)：HPLC grade.

3.1.2 Nitric acid (HNO_3)：Guarantee reagent.

3.2 Standard

Carrageenan：Food additive grade.

Konjac glucomannan：Food additive grade.

3.3 Preparation of standard solution

Standard stock solution（1.0 mg/mL）：Weigh about 50 mg carrageenan and konjac glucomannan standard material (accurate to 0.01 mg) in 50 mL volumetric flask, dissolve with water and dilute with water to a volume of 50 mL. The stock solution should be freshly prepared just before use.

3.4 Preparation of standard intermediate solution

Standard intermediate solution（10.0μg/mL）：Absorb standard stock solution1.00mL in 100 mL volumetric flask, dilute with water to a volume of 100 mL. The stock solution should be freshly prepared just before use.

4 Apparatus and equipment

4.1 High Performance Liquid Chromatography-Mass Spectrometer equipment: equipped with electrospray ionization source (ESI).

4.2 Analytical balance: sensibility reciprocal is 0.0001 g and 0.01 g respectively.

4.3 Centrifuge: ≥8000r/min.

4.4 Vortex mixer.

4.5 Tissue blender.

4.6 Homogenizer.

4.7 Thermostatic waterbath,

5 Procedure

5.1 Sample preparation and storage

About 100 g of representative samples should be taken from all samples, then homogenized by the homogenizer, put in suitable clean container. After being sealed and labeled, the samples should be stored at below -18℃ in dark. Certain measures should be taken to prevent contamination of samples or decomposition of the residues during the sample preparation procedure.

5.2 Sample processing

Carrageenan：Accurately 10 g test sample (accurate to 0.01 g) was weighed into a 50 mL centrifuge tube and 23 mL water was added, and then homogenized for 2 min at 10000rpm. 2 mL nitric acid was added. After vortex for 1 min, the mixture was incubated in thermostatic waterbath for 30 min at 85℃. After cooling too room temperature, the mixture was centrifuged for 5 min at 12000r/min. The upper layer was mixed with equivalent acetonitrile and filtered on a 0.22 μm filter prior to LC-MS/MS analysis.

Konjac glucomannan：Accurately 10 g test sample (accurate to 0.01 g) was weighed into a 50 mL centrifuge tube and 23 mL water was added, and then homogenized for 2

min at 10000rpm. 2 mL hydrochloric acid was added. After vortex for 1 min, the mixture was incubated in thermostatic waterbath for 30 min at 85℃. After cooling too room temperature, the mixture was centrifuged for 5 min at 12000r/min. The upper layer was mixed with equivalent acetonitrile and filtered on a 0.22 μm filter prior to LC-MS/MS analysis.

5.3 Preparation of matrix-based standard working curve

Carrageenan：Accurately 10 g test sample (accurate to 0.01 g) was weighed into a 50 mL centrifuge tube and standard stock solution was added separately, and then water was added to the volume of 23 mL. The sample was homogenized for 2 min at 10000rpm. 2 mL nitric acid was added. After vortex for 1 min, the mixture was incubated in thermostatic waterbath for 30 min at 85℃. After cooling too room temperature, the mixture was centrifuged for 5 min at 12000r/min. The upper layer was mixed with equivalent acetonitrile and filtered on a 0.22 μm filter prior to LC-MS/MS analysis.

Konjac glucomannan：Accurately 10 g test sample (accurate to 0.01 g) was weighed into a 50 mL centrifuge tube and standard stock solution was added separately, and then water was added to the volume of 23 mL. The sample was homogenized for 2 min at 10000rpm. 2 mL hydrochloric acid was added. After vortex for 1 min, the mixture was incubated in thermostatic waterbath for 30 min at 85℃. After cooling too room temperature, the mixture was centrifuged for 5 min at 12000r/min. The upper layer was mixed with equivalent acetonitrile and filtered on a 0.22 μm filter prior to LC-MS/MS analysis.

5.4 Apparatus operating condition

5.4.1 HPLC operating condition

a）Column: HILIC Plus，4.6×100mm×3.5μm or equivalent.

b）Column temperature：30℃；

c）Injection volume：5 μL；

d）Mobile phase: see Table 1.

Table 1 Mobile phase and gradient elution program

Time /min	Mobile phase A（water），%	Mobile phase B（acetonitrile），%
0	20	80
3	80	20
5	80	20
5.5	20	80

e）Flow rate：0.4 mL/min；

5.4.2 MS/MS operating condition

lon source：electrospray ionization source（ESI）；Scan mode：negative-ion mode；Monitor mode：multiple reaction monitoring (MRM)；Spray voltage：-3500V；Collision gas pressure：9 psi，Main MS parameters of target compound are listed in Table 2.

Table 2 Multiple reaction monitoring (MRM) parameters

Compound	Parent ion	Product ion	Declustering potential / V	Collision energy/ V
Carrageenan	403.0	241.0*, 97.0	120	30, 30
Konjac glucomannan	539.2	160.8*, 179.1	135	5,5

Note：* The product ion is used for quantification

5.5 Qualitative determination

Under the same determination conditions the variation range of the retention time for the peak of analyte in unknown sample and in the standard working solution can not be out of range of ±2.5% The variation range of the ion ratio between the qualitative ion for the unknown sample and the standard working solution at the similar concentration can not be out of range of Table 3. Then the corresponding analyte can be present in the sample.

Table 3 Maximum permitted tolerances for relative ion intensities

Relative intensity（%）	>50	>20~50	>10~20	≤10
Maximum permitted tolerances for	±20	±25	±30	±50

relative ion intensities（%）				

5.6 Quantitation determination

Under the optimized instrument working conditions, different matrix-based working standard solutions were injected. Using peak area as y-axis and the concentration as x-axis, the concentration of carrageenan in sample is quantified by standard calibration curve. The response of the carrageenan in the sample solution should be in the linear range of the instrument detection.

5.7 Determination of sample solution

Using the sample solution in Part 5.2, the mass concentration of carrageenan in the sample was calculated by using the matrix-based external standard method.

6 Blank test

The operation of the blank test is the same as that described in the method of determination but with omission of sample addition.

7 Calculation and expression of the result

The calculation of carrageenan and konjac glucomannan in the sample is according to Formula (1)

$$X = \frac{c \times v}{m} \times \mathrm{f} \quad \text{.....(1)}$$

where：

X—the content of analyte in the test sample, mg/kg；

c—the concentration of analyte which is quantified by standard calibration curve, μg/mL；

v—the final volume of sample solution, mL；

m—the corresponding mass of test sample, g；

f—dilution ratio of sample solution.

The result was expressed as the arithmetic mean of two independent determinations obtained under repeatability conditions and rounded to two decimal places.

8 Precision

The absolute difference of two independent determinations obtained under

repeatability conditions shall not exceed 10% of the arithmetic mean.

9 Limit of quantitation

When sample weight is 10 g，the limit of determination of carrageenan is 25μg/kg and the limit of quantification is 100μg/kg.

10 Recovery

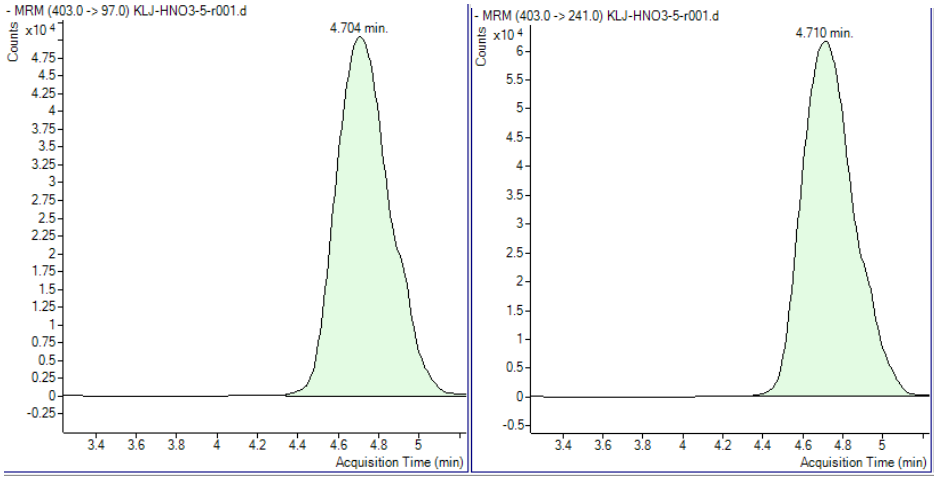
The recoveries of carrageenan in pork and prawn:

Spiked:200μg/kg、2.0mg/kg、10.0 mg/kg，recovery:81.62~113.7%.

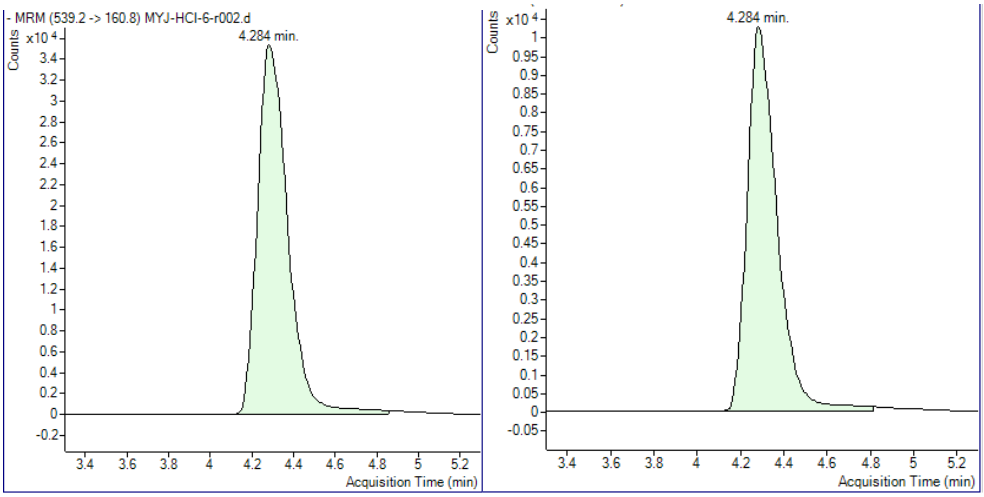
Annex A

(Informative)

LC-MS/MS chromatogram of carrageenan hydrolyzate



MRM chromatogram of carrageenan hydrolyzate



MRM chromatogram of konjac glucomannan hydrolyzate