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

GB 31656.14-2022

**National food safety standard - Determination of 27 sex
hormones residues in fishery products by liquid
chromatography-tandem mass spectrometric**

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6.2 Analytical balance. Sensitivity 0.00001g and 0.01g.

6.3 High speed centrifuge. 10000r/min.

6.4 Vortex mixer.

6.5 Homogenizer.

6.6 Rotary evaporator.

6.7 Solid phase extraction device.

6.8 Pear-shaped bottle. 100mL.

6.9 Ultrasonic cleaner.

6.10 Plastic centrifuge tubes. 10mL and 50mL.

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4.0 µg/kg.

10.2 Accuracy

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1. Structure and Drafting Rules for Standardization Documents" drafting.

This document is published for the first time.

National Food Safety Standards

Determination of 27 sex hormone residues in aquatic products by liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry method for the detection of 27 sex hormone residues in aquatic products.

This document applies to diethylstilbestrol, diethylstilbestrol, estrone, hexestrol, estradiol, estriol, ethinyl estradiol,

Estradiol Benzoate, Trenbolone, Nandrolone, Androstenedione, Boldenone, Testosterone, Norethindrone, Medrosterone, Methyltestosterone, Stanozolol, Nandrolone Phenylpropionate, Propionate

testosterone, progesterone, 21alpha-hydroxyprogesterone, 17alpha-hydroxyprogesterone, medroxyprogesterone, megestrol acetate, chlormadinone acetate, medroxyprogesterone acetate and levoprogesterone

Determination of norgestrel residues.

2 Normative references

The content in the following documents constitutes the essential provisions of this document through normative references in the text. Among them, the dated reference documents are only

The version corresponding to the date applies to this document; for undated references, the

latest version (including all amendments) applies to this document.

GB/T 6682 Analytical laboratory water specifications and test methods

GB/T 30891-2014 Sampling Specifications for Aquatic Products

3 Terms and Definitions

This document does not have terms and definitions that need to be defined.

4 principles

The remaining sex hormones in the sample were extracted sequentially with ethyl acetate-methyl tert-butyl ether mixed solvent and ethyl acetate, the extract was concentrated, and C18 solid

It was purified by phase extraction column, determined by liquid chromatography-tandem mass spectrometer, and quantified by internal standard method.

5 Reagents and materials

The reagents used below are analytically pure unless otherwise specified; the water is the first-grade water in accordance with the provisions of GB/T 6682.

5.2 Solution preparation

5.2.1 Acetic acid-sodium acetate buffer solution. Take 21.5g of sodium acetate trihydrate and 2.6mL of glacial acetic acid, add appropriate amount of water to dissolve and dilute to 500mL,

mix well.

5.2.2.0.02% ammonia water. Take 100 μ L of ammonia water in 500 mL of water, mix well, and prepare before use.

5.2.3 Ethyl acetate-methyl tert-butyl ether solution. Mix equal volumes of ethyl acetate and methyl tert-butyl ether.

5.3 Standards

5.3.1 Sex hormones. diethylstilbestrol, diethylstilbestrol, estrone, hexestrol, estradiol, estriol, ethinylestradiol, estradiol benzoate, trenbolone,

Nandrolone, Androstenedione, Boldenone, Testosterone, Norethindrone, Meandrosterone, Methyltestosterone, Stanozolol, Nandrolone Phenylpropionate, Testosterone Propionate, Progesterone, 21alpha-hydroxy

Progesterone, 17alpha-hydroxyprogesterone, medroxyprogesterone, megestrol acetate,

chlormadinone acetate, medroxyprogesterone acetate and levonorgestrel. content $\geq 97\%$,

See Appendix A for details.

5.3.2 Internal standard. diethylstilbestrol-D8, estrone-D2, estradiol-13C2, methyltestosterone-D3, progesterone-D9, levonorgestrel-D6 and medroxyprogesterone-D3.

Content $\geq 97\%$, see Appendix A for details.

5.4 Preparation of standard solution

5.4.1 Standard stock solution. Take 10mg of sex hormone standard products, weigh them accurately, dissolve them in appropriate amount of methanol and dilute to 100mL.

Color volumetric flasks were used to prepare standard stock solutions with a concentration of 100 $\mu\text{g/mL}$. Stored below -18°C , the validity period was 1 year.

5.4.2 Mixed standard intermediate solution. Accurately measure the appropriate amount of each standard stock solution, put it in a 100mL brown volumetric flask, dilute it with methanol to prepare hexadiene

Diethylstilbestrol, Diethylstilbestrol, Estrone, Trenbolone, Nandrolone, Androstenedione, Boldenone, Testosterone, Meandrosterone, Methyltestosterone, Nandrolone Phenylpropionate, Testosterone Propionate,

The concentrations of progesterone, 21alpha-hydroxyprogesterone, 17alpha-hydroxyprogesterone, medroxyprogesterone, megestrol acetate, chlormadinone acetate and medroxyprogesterone acetate were all

1 $\mu\text{g/mL}$; the concentrations of estradiol, levonorgestrel, norethindrone, estradiol benzoate and stanozolol were all 2 $\mu\text{g/mL}$; estriol and

The concentration of ethinyl estradiol was 4 $\mu\text{g/mL}$, and the mixed standard intermediate solution was stored below -18°C , and the validity period was 6 months.

5.4.3 Mixed standard working solution. Accurately measure 10mL mixed standard intermediate solution, put it in a 50mL brown volumetric flask, dilute it to the mark with methanol,

Diethylstilbestrol, Diethylstilbestrol, Estrone, Trenbolone, Nandrolone, Androstenedione, Boldenone, Testosterone, Meandrosterone, Methyltestosterone, Phenylpropionate

Nandrolone, testosterone propionate, progesterone, 21alpha-hydroxyprogesterone, 17alpha-hydroxyprogesterone, medroxyprogesterone, megestrol acetate, chlormadinone acetate, and medroxyprogesterone acetate

The concentrations of progesterone were all 0.2 $\mu\text{g/mL}$; the concentrations of hexestrol, estradiol, levonorgestrel, norethindrone, estradiol benzoate and stanozolol were all