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Method of Test for Estrogens in Cosmetics

1. Scope

This method is applicable to the determination of 8 estrogens (estriol, estradiol, estrone, ethinyl estradiol, estradiol benzoate, diethylstilbestrol, pregnanediol and progesterone) in cosmetics.

2. Method

After extraction, analytes are determined by liquid chromatography/ tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: ACQUITY UPLC BEH C18, 1.7 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Methanol, HPLC grade;

Acetonitrile, HPLC grade;

Deionized water, resistivity ≥ 18 MΩ・cm (at 25℃);

Estriol, estradiol, estrone, ethinyl estradiol, estradiol benzoate, diethylstilbestrol, pregnanediol and progesterone, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL, 20 mL and 50 mL.

2.3.2. Membrane filter: 0.22 μm, Nylon.

2.4. Mobile phase

2.4.1. Solvent A: Deionized water.

2.4.2. Solvent B: Acetonitrile.

2.5. Standard solution preparation

Transfer about 5 mg of estrogen reference standards accurately weighed into each 50 mL volumetric flask, dissolve and dilute with methanol to the volume as the standard stock solutions. Store at 4℃. When to use, mix appropriate volume of each standard stock solution, and dilute with methanol to 0.005-1.0 μg/mL as the standard solutions.

2.6. Sample solution preparation

Transfer about 1 g of the well-mixed sample accurately weighed into a 20 mL volumetric flask. Add 15 mL of methanol, and ultrasonicate

for 30 min. Add methanol to the volume. Filter with a membrane filter, and take the filtrate as the sample solution.

2.7. Standard curve establishment

Accurately inject 5 μL of each the standard solution into LC-MS/MS separately, and operate according to the following conditions. Establish the standard curve of each estrogen by the peak area of each estrogen vs. the corresponding concentrations of each estrogen.

LC-MS/MS operating conditions (note):

Column: ACQUITY UPLC BEH C18, 1.7 μm, 2.1 mm i.d. × 10 cm.

Column temperature: 30℃.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0.0 → 4.0	50 → 30	50 → 70
4.0 → 5.0	30 → 10	70 → 90
5.0 → 7.0	10 → 10	90 → 90
7.0 → 7.5	10 → 50	90 → 50
7.5 → 8.0	50 → 50	50 → 50

Flow rate: 0.3 mL/min.

Injection volume: 5 μL.

Capillary voltage: 3.98 kV.

Ion source temperature: 150℃.

Desolvation temperature：350℃.

Detection mode: multiple reaction monitoring (MRM). Selected ion pair, cone voltage (CV) and collision energy (CE) are as follows.

Analyte	Ionization mode	Ion pair	Cone voltage (V)	Collision energy (eV)
		Precursor ion (*m/z*) > product ion (*m/z*)		
Estriol	ESI^-	287 > 171*	26	42
		287 > 145		36
Estradiol	ESI^-	271 > 145*	78	44
		271 > 183		50
Estrone	ESI^-	269 > 145*	66	42
		269 > 159		40
Ethinyl estradiol	ESI^-	295 > 145*	68	48

		295 > 159		42
Diethylstilbestrol	ESI^-	267 > 237*	52	34
		267 > 251		32
Pregnanediol	ESI^+	285 > 189*	30	34
		285 > 175		28
Progesterone	ESI^+	315 > 109*	40	44
		315 > 123		28
Estradiol benzoate	ESI^+	377 > 135*	38	26
		377 > 105		26

*Quantitative ion pair

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.8. Identification and quantification

Accurately inject 5 μL of the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.7. Identify each estrogen based on the retention time and the relative ion intensities(note) by multiple reaction monitoring. Calculate the amount of each estrogen in the sample by the following formula:

$$\text{The amount of each estrogen in the sample (ppm)} = \frac{C \times V}{M}$$

Where,

C: the concentration of each estrogen in the sample solution calculated by the standard curve (μg/mL)

V: the final make-up volume of the sample (mL)

M: the weight of sample (g)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions ($\leq$ 100%). Maximum permitted tolerances of relative ion intensities by LC-MS/MS are as follows.

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≤ 10	± 50

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Remark

1. Limits of quantitation (LOQs) for estriol, estradiol, estrone, ethinyl estradiol, estradiol benzoate, diethylstilbestrol and progesterone are 0.1 ppm, and pregnanediol is 1.0 ppm.
2. Further validation should be performed when interfering compounds are found in samples.