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Method of Test for Prostaglandin Analogs in Cosmetics

1. Scope

This method is applicable to the determination of 8 prostaglandin analogs (bimatoprost, etc. listed in the attached table) 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: Kinetex XB-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;

Formic acid, HPLC grade;

Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);

Bimatoprost, dechloro dihydroxy difluoro ethylcloprostenolamide, cloprostenol, ethyl travoprostamide, fluprostenol, latanoprost, travoprost and tafluprost, reference standards.

Bimatoprost-d_5, fluprostenol-d_4, travoprost-d_4 and latanoprost-d_4, isotope labeled internal standard.

2.3. Apparatus

2.3.1. Volumetric flask: 5 mL and 10 mL, Pyrex.

2.3.2. Membrane filter: 0.22 μm, PVDF.

2.4. Mobile phase

2.4.1. Solvent A:

Dilute 1 mL of formic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.4.2. Solvent B:

Dilute 1 mL of formic acid with methanol to 1000 mL, and filter with a membrane filter.

2.5. Internal standard solution preparation

Transfer about 1 mg of 4 isotope labeled internal standards (bimatoprost-d_5, etc.) accurately weighed into each 5 mL volumetric flask, dissolve and dilute to volume with methanol as the internal standard stock solutions. Store at -20°C in the dark. When to use, mix appropriate amount of each internal standard stock solution, and dilute with methanol to 10 μg/mL as the internal standard solution.

2.6. Standard solution preparation

Transfer about 1 mg of 8 reference standards (bimatoprost, etc.) into each 10 mL volumetric flask, dissolve and dilute with methanol to the volume as the standard stock solutions. Store at -20°C in the dark. When to use, mix appropriate volume of each standard stock solution, and dilute with methanol to 1-20 ng/mL for bimatoprost, latanoprost, dechloro dihydroxy difluoro ethylcloprostenolamide and ethyl travoprostamide, to 5-100 ng/mL for cloprostenol, fluprostenol, tafluprost and travoprost (all of them containing 10 ng/mL isotope labeled internal standards), as the standard solutions.

2.7. Standard curve preparation

Accurately inject 3 μL of each the standard solution into LC-MS/MS separately, and operate according to the following conditions. Establish the standard curve of each prostaglandin analog by the ratios of the peak area of each prostaglandin analog to that of the isotope labeled internal standard vs. the concentrations of each prostaglandin analog.

LC-MS/MS operating conditions (note 1):

Column: Kinetex XB-C18, 1.7 μm, 2.1 mm i.d. × 10 cm.

Column temperature: 40°C.

Sample tray temperature: 10°C.

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

Time (min)	Solvent A (%)	Solvent B (%)
0.0 → 1.0	80 → 80	20 → 20
1.0 → 4.0	80 → 0	20 → 100
4.0 → 6.0	0 → 0	100 → 100
6.0 → 6.5	0 → 80	100 → 20
6.5 → 12.0	80 → 80	20 → 20

Flow rate: 0.3 mL/min.

Injection volume: 3 μL.

Capillary voltage: ESI^+, 2.5 kV; ESI^-, 2.5 kV.
Ion source temperature: 150°C.
Desolvation temperature: 400°C.
Cone gas flow rate: 150 L/hr.
Desolvation flow rate: 870 L/hr.
Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage and collision energy are as the attached table.

Note 1: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.8. Sample solution preparation

Transfer about 0.5 g of the well-mixed sample accurately weighed into a 10 mL volumetric flask, add 8 mL of methanol and 10 μL of internal standard solution. Ultrasonicate for 15 min. Dilute to volume with methanol and filter with a membrane filter. Take the filtrate as the sample solution.

2.9. Identification and quantification

Accurately inject 3 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 prostaglandin analog based on the retention time and the relative ion intensities (note 2). Calculate the amount of each prostaglandin analog (μg/g) in the sample by the following formula:

$$\text{The amount of each prostaglandin analog in the sample (μg/g)} = \frac{C \times V}{M} \times 10^{-3}$$

where,

C: the concentration of each prostaglandin analog in the sample solution calculated by the standard curve (ng/mL)
V: the final make-up volume of sample (mL)
M: the weight of sample (g)

Note 2: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤100%). Maximum permitted tolerances for relative ion intensities by LC-MS/MS are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20

> 20 - 50	± 25
> 10 - 20	± 30
≤ 10	± 50

Remark

1. Limits of quantification (LOQs) of prostaglandin analogs are listed in the attached table.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Wittenberg, J. B., Zhou, W., Wang, P. G. and Krynitsky, A. J. 2014. Determination of prostaglandin analogs in cosmetic products by high performance liquid chromatography with tandem mass spectrometry. J. Chromatogr. A 1359: 140-146.
2. Marchei, E., De Orsi, D., Guarino, C., Rotolo, M. C., Graziano, S. and Pichini, S. 2016. High performance liquid chromatography tandem mass spectrometry measurement of bimatoprost, latanoprost and travoprost in eyelash enhancing cosmetic serums. Cosmetics 3: 4.

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Reference chromatogram

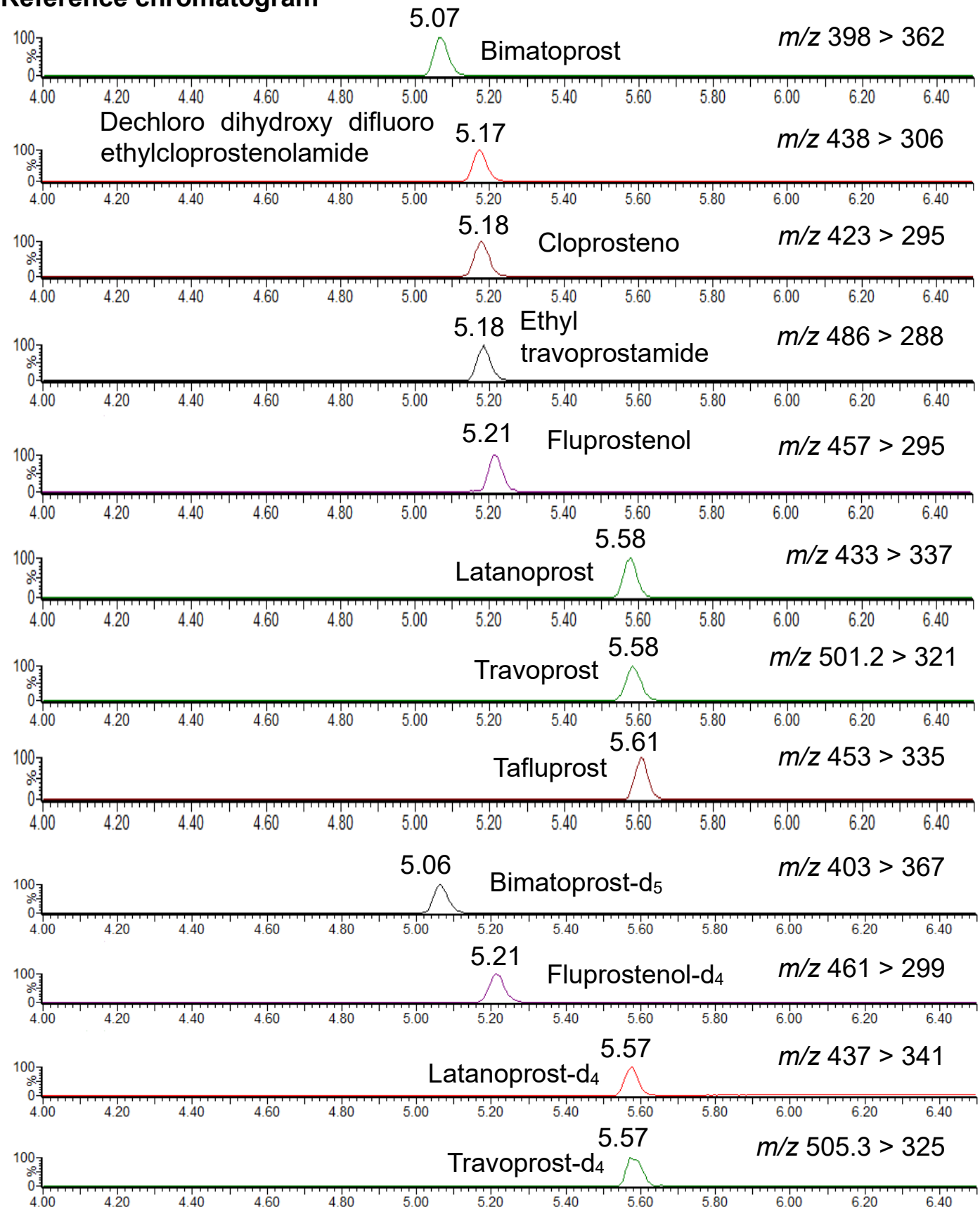

Figure. MRM chromatograms of 8 prostaglandin analogs standards and 4 isotope labeled internal standards analyzed by LC-MS/MS

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Table. MRM parameters and limits of quantification (LOQs) of 8 prostaglandin analogs.

No	Analyte	Ionization mode	Ion pair: Precursor ion (*m/z*) > product ion (*m/z*)	cone voltage (V)	collision energy (eV)	Internal standard	LOQs (μg/g)
1	Bimatoprost	ESI^+	398 > 362* 398 > 317	20	10 15	Bimatoprost-d_5	0.02
2	Dechloro dihydroxy difluoro ethylcloprostenolamide	ESI^+	438 > 306* 438 > 232	25	10 14	Bimatoprost-d_5	0.02
3	Cloprostenol	ESI^-	423 > 295* 423 > 259	25	15 15	Fluprostenol-d_4	0.1
4	Ethyl travoprostamide	ESI^+	486 > 288* 486 > 234	25	8 12	Bimatoprost-d_5	0.02
5	Fluprostenol	ESI^-	457 > 295* 457 > 259	40	20 15	Fluprostenol-d_4	0.1
6	Latanoprost	ESI^+	433 > 337* 433 > 379	10	15 10	Latanoprost-d_4	0.02
7	Travoprost	ESI^+	501.2 > 321* 501.2 > 249	8	8 11	Travoprost-d_4	0.1
8	Tafluprost	ESI^+	453 > 335* 453 > 261	20	12 14	Travoprost-d_4	0.1
I.S.	Bimatoprost-d_5	ESI^+	403 > 367	15	8	-	-
I.S.	Fluprostenol-d_4	ESI^-	461 > 299	35	18	-	-
I.S.	Latanoprost-d_4	ESI^+	437 > 341	10	16	-	-
I.S.	Travoprost-d_4	ESI^+	505.3 > 325	20	4	-	-

*Quantitative ion pair.