

Method of Test for Preservatives in Cosmetics (4)

1. Scope

This method is applicable to the determination of 8 preservatives (chlorhexidine, etc. listed in Table 1) in cosmetics.

2. Method

After extraction, preservatives 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 BEH Shield RP18 , 1.7 μm , 2.1 mm i.d. $\times$ 10 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.1.3. Vortex mixer.

2.2. Chemicals

Methanol, LC-MS grade (Honeywell, or an equivalent);

Acetonitrile, HPLC grade;

Deionized water, resistivity $\geq 18 \text{ M}\Omega \cdot \text{cm}$ (at 25°C);

Chlorhexidine and other preservatives are listed in Table 1, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL and 50 mL.

2.3.2. Membrane filter: 0.22 μm , Nylon.

2.4. Reagent

2.4.1 Methanol: acetonitrile (3:1, v/v) solution

Mix methanol and acetonitrile at the ratio of 3:1 (v/v).

2.4.2 Extraction solution

Mix solvent A and solvent B at the ratio of 1:1 (v/v).

2.5. Mobile phase

2.5.1. Solvent A

Dilute 8 mL of formic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B

Dilute 8 mL of formic acid with methanol: acetonitrile (3:1, v/v) solution to 1000 mL, and filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 10 mg of reference standards (chlorhexidine, etc) accurately weighed into each 10-mL volumetric flask, dissolve and dilute with extraction solution to volume as the standard stock solutions. Store in a refrigerator. Upon use, mix appropriate volume of each standard stock solution, and dilute with extraction solution as the standard solutions. The concentration range of each standard solution is listed in Table 1.

2.7. Sample solution preparation

2.7.1. General cosmetics

Transfer about 0.5 g of the well-mixed sample accurately weighed into a 50-mL volumetric flask. Add 40 mL of extraction solution, and sonicate for 30 min. Add extraction solution to volume. Filter with a membrane filter, and take the filtrate as the sample solution.

2.7.2. Mouthwash and baby wipes (for analysis of chlorhexidine)

2.7.2.1. Mouthwash

Transfer about 1.0 g of the well-mixed sample accurately weighed into a 10-mL volumetric flask. Add 8 mL of extraction solution, and sonicate for 30 min. Add extraction solution to volume. Filter with a membrane filter, and take the filtrate as the sample solution.

2.7.2.2. Baby wipes

Transfer about 1.0 g of the liquid part of the sample accurately weighed into a 10-mL volumetric flask. Add 8 mL of extraction solution, and sonicate for 30 min. Add extraction solution to volume. Filter with a membrane filter, and take the filtrate as the sample solution.

2.8. Identification and quantification

Accurately inject 1 μ L of the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the following conditions. Identify each preservative based on the retention time and the relative ion intensities ^(note 1). Calculate the amount (%) of each preservative in the sample by the following formula:

$$\text{The amount of each preservative in the sample (\%)} = \frac{C \times V}{M} \times 10^{-4}$$

where,

C: the concentration of each preservative in the sample solution calculated by the standard curve (μ g/mL)

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

M: the weight of sample (g)

LC-MS/MS operating conditions ^(note 2):

Column: ACQUITY BEH Shield RP18, 1.7 μ m, 2.1 mm i.d. $\times$ 10 cm.

Column temperature: 40°C.

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

Time (min)	A (%)	B (%)
0.0 $\rightarrow$ 4.0	90 $\rightarrow$ 40	10 $\rightarrow$ 60
4.0 $\rightarrow$ 6.0	40 $\rightarrow$ 40	60 $\rightarrow$ 60
6.0 $\rightarrow$ 8.0	40 $\rightarrow$ 10	60 $\rightarrow$ 90
8.0 $\rightarrow$ 11.0	10 $\rightarrow$ 10	90 $\rightarrow$ 90
11.0 $\rightarrow$ 12.0	10 $\rightarrow$ 90	90 $\rightarrow$ 10
12.0 $\rightarrow$ 15.0	90 $\rightarrow$ 90	10 $\rightarrow$ 10

Flow rate: 0.3 mL/min.

Injection volume: 1 μ L.

Capillary voltage: 3.00 kV.

Ion source temperature: 150°C.

Desolvation temperature: 500°C.

Cone gas flow rate: 30 L/hr.

Desolvation flow rate: 600 L/hr.

Detection mode: multiple reaction monitoring (MRM). Selected ion pair, cone voltage (CV) and collision energy (CE) are shown in Table 2.

Note :1. Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions ($\leq$ 100%). Maximum permitted tolerances for relative ion intensities by LC-MS/MS are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	$\pm$ 20
> 20-50	$\pm$ 25
> 10-20	$\pm$ 30
$\leq$ 10	$\pm$ 50

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

Remark

1. Limits of quantitation (LOQs) of 8 preservatives are listed in Table 2.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Bukanski, B. W. and Masse, M. O. 1984. Analysis of hexamidine, dibromohexamidine, dibromopropamidine and chlorhexidine in cosmetic products by high-performance liquid chromatography. *Int. J. Cosmet. Sci.* 6: 283-292.
2. Liu, Y., Wang, H., Yang, H., Shi, H. and Guo, Q. 2011. Simultaneous determination of 3 benzalkonium chloride homologs in cosmetics by high performance liquid chromatography. *Chinese Journal of Chromatography* 29: 458-461.
3. Bukanski, B. W. 1987. Analysis of domiphen bromide and cetylpyridinium chloride in cosmetic products by high-performance liquid chromatography. *Int. J. Cosmet. Sci.* 9: 193-198.
4. Terol, A., Gómez-Mingot, M., Maestre, S. E., Prats, S., Luis Todolí, J. and Paredes, E. 2010. Simple and rapid analytical method for the simultaneous determination of cetrimonium chloride and alkyl alcohols in hair conditioners. *Int. J. Cosmet. Sci.* 32: 65-72.

Reference chromatograms

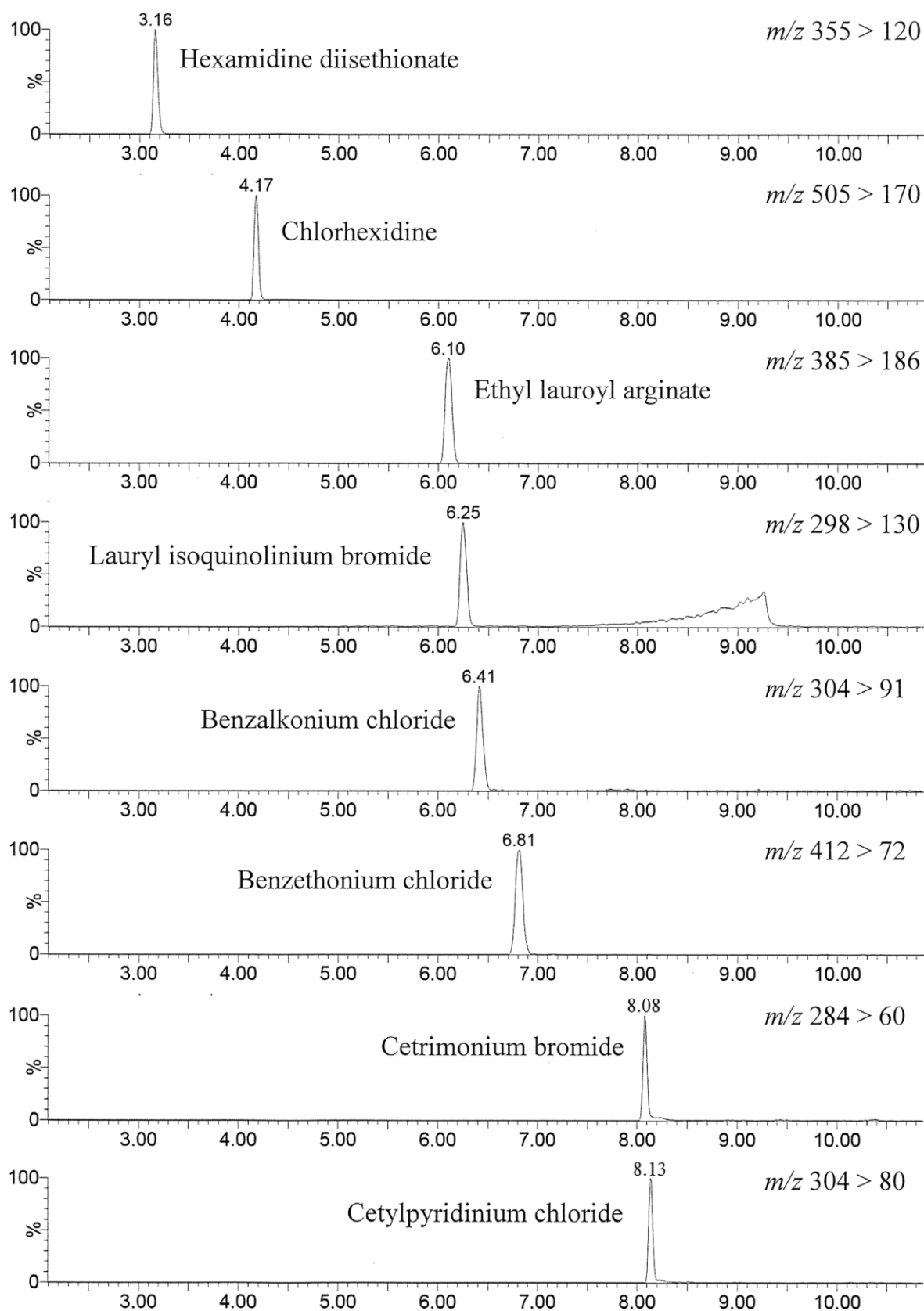


Figure. MRM chromatograms of 8 preservatives standards analyzed by LC-MS/MS.

Table 1. Concentration ranges of preservatives.

Analyte	Concentration range (µg/mL)
Benzalkonium chloride	0.01-0.2
Benzethonium chloride	0.01-0.2
Cetylpyridinium chloride	0.01-0.2
Lauryl isoquinolinium bromide	0.01-0.2
Cetrimonium bromide	0.01-0.2
Ethyl lauroyl arginate	0.01-0.2
Hexamidine diisethionate	2-20
Chlorhexidine	2-20

Table 2. MRM parameters and LOQs of preservatives.

Analyte	Ionization mode	Ion pair		Cone voltage (V)	Collision energy (eV)	Sample type	Limits of quantitation (%)
		Precursor ion (m/z) >	Product ion (m/z)				
Hexamidine diisethionate	ESI ⁺	355 > 120*	355 > 338	40	50 18	General cosmetics	0.02
Chlorhexidine	ESI ⁺	505 > 170*	505 > 125	32	36 40	Mouthwash and baby wipes General cosmetics	0.002 0.02
Ethyl lauroyl arginate	ESI ⁺	385 > 186*	385 > 70	40	24 42	General cosmetics	0.0001
Lauryl isoquinolinium bromide	ESI ⁺	298 > 130*	298 > 57	48	26 26	General cosmetics	0.0001
Benzalkonium chloride	ESI ⁺	304 > 91*	304 > 58	44	24 28	General cosmetics	0.0001
Benzethonium chloride	ESI ⁺	412 > 72*	412 > 91	42	24 38	General cosmetics	0.0001
Cetrimonium bromide	ESI ⁺	284 > 60*	284 > 57	50	24 24	General cosmetics	0.0001
Cetylpyridinium chloride	ESI ⁺	304 > 80*	304 > 57	50	28 26	General cosmetics	0.0001

*quantitative ion pair.