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Method of Test for Colorants in Cosmetics (2)

1. Scope

This method is applicable to the determination of 12 colorants (pigment orange 13, etc. listed in the table 1) 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 HSS T3, 1.8 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Isopropanol, HPLC grade;
Acetonitrile, HPLC grade;
Dimethyl sulfoxide (DMSO), HPLC grade;
Xylene, HPLC grade;
Chloroform, HPLC grade;
Ammonium formate, GR grade;
Ammonia water (25%), GR grade;
Deionized water, resistivity ≥ 18 MΩ・cm (at 25°C);
Pigment orange 13 and other colorants listed in the table 1, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL and 20 mL.

2.3.2. Membrane filter: 0.22 μm, PTFE.

2.4. Extraction solution

Mix DMSO, xylene, chloroform at the ratio of 7:2:1 (v/v/v).

2.5. Mobile phase

2.5.1. Solvent A:

Dissolve and dilute 0.63 g of ammonium formate with deionized water to 1000 mL. Adjust pH to 9 with ammonia water, and filter with a membrane filter.

2.5.2. Solvent B:

Mix isopropanol and acetonitrile at the ratio of 2:8 (v/v), and filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 10 mg of reference standards accurately weighed into each 10 mL volumetric flask, dissolve and dilute with appropriate solvents listed in the table 1 to the 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 the table 1.

2.7. Sample solution preparation

Transfer about 1 g of the well-mixed sample accurately weighed into a 20 mL volumetric flask. Add 15 mL of extraction solution, and sonicate for 30 min. Add extraction solution to the volume. Filter with a membrane filter, and take the filtrate as the sample solution.

2.8. 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 following conditions. Identify each colorant based on the retention time and the relative ion intensities (note1) by multiple reaction monitoring. Calculate the amount of each colorant in the sample by the following formula:

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

where,

C: the concentration of each colorant in the sample solution calculated by the standard curve (µg/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 HSS T3, 1.8 µm, 2.1 mm i.d. × 10 cm.
Column temperature: 40℃.
Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0 → 3	90 → 40	10 → 60
3 → 6	40 → 0	60 → 100
6 → 14	0 → 0	100 → 100

14 → 14.5	0 → 90	100 → 10
14.5 → 16	90 → 90	10 → 10

Flow rate: 0.4 mL/min.

Injection volume: 3 μL.

Capillary voltage: 2.9 KV.

Ion source temperature: 150℃.

Desolvation temperature: 500℃.

Cone gas flow rate: 30 L/hr.

Desolvation flow rate: 650 L/hr.

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

Note: 1. 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

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 colorants are listed in the Table 2.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Guerra, E., Celeiro, M., Lamas, J. P., Llompart, M. and Garcia-Jares, C. 2015. Determination of dyes in cosmetic products by micro-matrix solid phase dispersion and liquid chromatography coupled to tandem mass spectrometry. J. Chromatogr. A 1415: 27-37.
2. Feng, F., Zhao, Y., Yong, W., Sun, L., Jiang, G. and Chu, X. 2011. Highly sensitive and accurate screening of 40 dyes in soft drinks by liquid

chromatography–electrospray tandem mass spectrometry. J. Chromatogr. B 879: 1813-1818.

Reference chromatograms

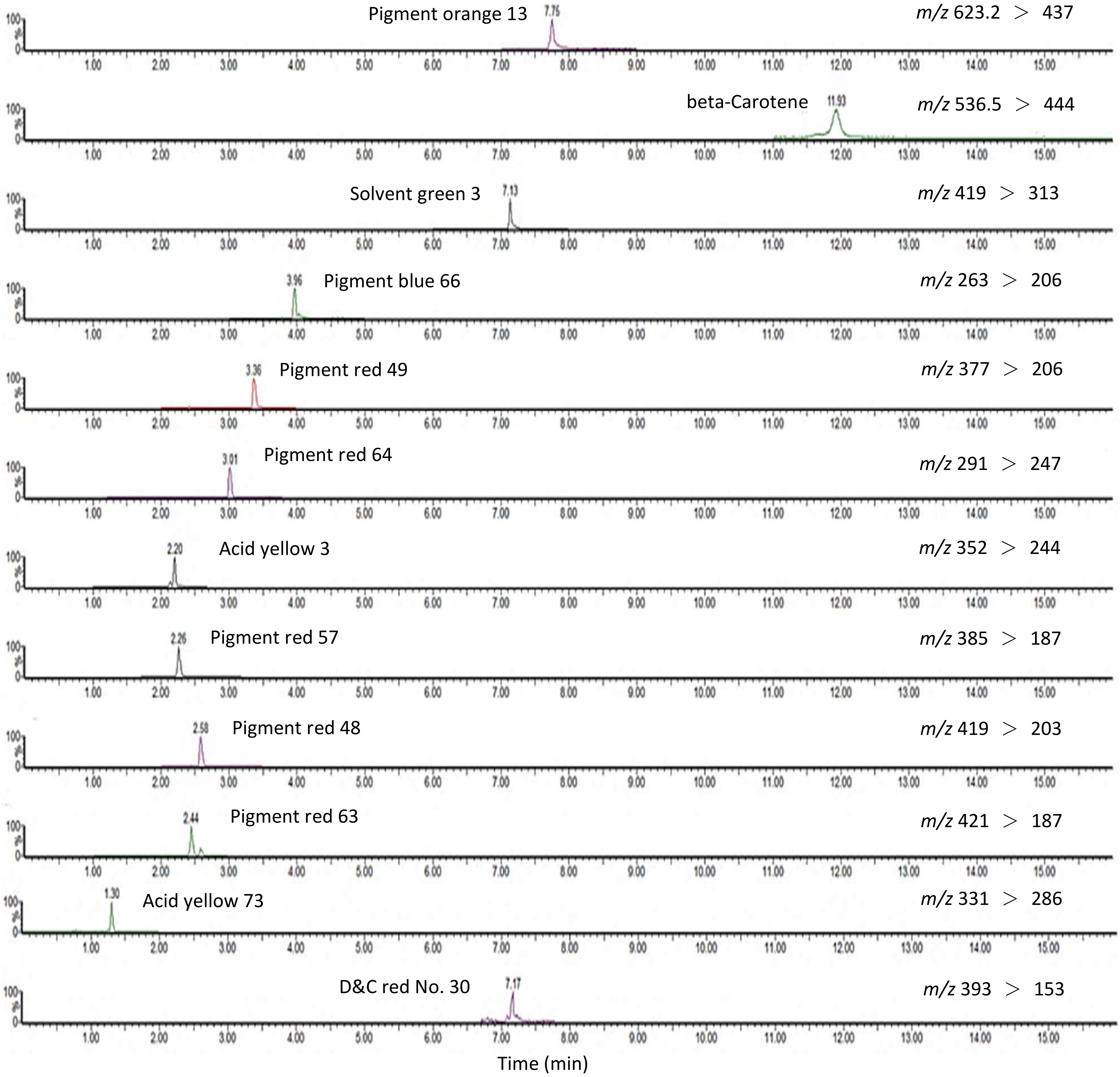

Figure. MRM chromatograms of 12 colorants analyzed by LC-MS/MS.

Table 1. Concentration ranges of 12 colorant standard solutions

Item	Analyte	Color index number	Solvent	Concentration range (μg/mL)
1	Pigment orange 13	CI 21110	Chloroform	0.01～0.1
2	beta-Carotene	CI 40800	DMSO	
3	Solvent green 3	CI 61565	DMSO	
4	Pigment blue 66	CI 73000	DMSO	
5	Pigment red 49	CI 15630	DMSO	0.2～2.0
6	Pigment red 64	CI 15800	DMSO	
7	Acid yellow 3	CI 47005	Methanol	
8	Pigment red 57	CI 15850	DMSO	0.5～5.0
9	Pigment red 48	CI 15865	DMSO	
10	Pigment red 63	CI 15880	DMSO	
11	Acid yellow 73	CI 45350	Methanol	
12	D&C red No. 30	CI 73360	Xylene	

Table 2. MRM parameters and LOQs of 12 colorants

Item	Analyte	Ionization mode	Ion pair Precursor ion (*m/z*) ＞ Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Limit of quantitation (%)
1	Pigment orange 13	ESI$^+$	623.2 ＞ 437*	70	26	0.00002
			623.2 ＞ 250		46	
2	beta-Carotene	ESI$^+$	536.5 ＞ 444*	36	16	0.00002
			536.5 ＞ 105		66	
3	Solvent green 3	ESI$^+$	419 ＞ 313*	56	24	0.00002
			419 ＞ 401		26	
4	Pigment blue 66	ESI$^-$	263 ＞ 206*	50	20	0.00002
			263 ＞ 235		28	
5	Pigment red 49	ESI$^-$	377 ＞ 206*	48	28	0.0004
			377 ＞ 297		18	
6	Pigment red 64	ESI$^-$	291 ＞ 247*	26	14	0.0004
			291 ＞ 142		26	
7	Acid yellow 3	ESI$^-$	352 ＞ 244*	64	26	0.0004
			352 ＞ 259		38	
8	Pigment red 57	ESI$^-$	385 ＞ 187*	44	22	0.001
			385 ＞ 170		26	
9	Pigment red 48	ESI$^-$	419 ＞ 203*	50	16	0.001
			419 ＞ 139		30	
10	Pigment red 63	ESI$^-$	421 ＞ 187*	50	28	0.001
			421 ＞ 269		20	
11	Acid yellow 73	ESI$^-$	331 ＞ 286*	42	28	0.001
			331 ＞ 243		30	
12	D&C Red No.30	ESI$^+$	393 ＞ 153*	25	14	0.001
			393 ＞ 89		18	

*Quantitative ion pair