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Method of Test for Imperatorin、5-Methoxypsoralen、8-Methoxypsoralen、6-Methylcoumarin、Musk Ambrette、Safrole and Trioxysalen in Cosmetics

1. Scope

This method is applicable to the determination of imperatorin, 5-methoxypsoralen, 8-methoxypsoralen, 6-methylcoumarin, musk ambrette, safrole and trioxysalen in cosmetics.

2. Method

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

2.1. Equipment

2.1.1. Gas chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electron ionization, EI.

2.1.1.2. Column: HP-5MS UI capillary column, 0.25 μm, 0.25 mm i.d. × 30 m, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Acetone, HPLC grade;

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

Imperatorin, 5-methoxypsoralen, 8-methoxypsoralen, 6-methylcoumarin, musk ambrette, safrole and trioxysalen, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 20 mL.

2.3.2. Membrane filter: 0.22 μm, Nylon.

2.4. Standard solution preparation

Transfer about 20 mg of imperatorin, 5-methoxypsoralen, 8-methoxypsoralen, 6-methylcoumarin, musk ambrette, safrole and trioxysalen reference standards accurately weighed to each 20-mL volumetric flask, dissolve and dilute with acetone to volume as the standard stock solutions. When to use, mix appropriate amount of each standard stock solution, and dilute with acetone to 5 - 100 ng/mL for 5-methoxypsoralen, 8-methoxypsoralen, 6-methylcoumarin, safrole and trioxysalen, to 10 - 100 ng/mL for imperatorin and musk ambrette, as the standard solutions.

2.5. Sample solution preparation

Transfer about 1 g of the well-mixed sample accurately weighed into a 20-mL volumetric flask, add 10 mL of acetone and ultrasonicate for 30 mins. Dilute to volume with acetone and filter with a membrane filter. Take the filtrate as the sample solution.

2.6. Identification and quantification

Accurately inject 2 μL of the sample solution and the standard solutions into GC-MS/MS separately, and operate according to the following conditions. Identify each analyte based on the retention time and the relative ion intensities(note 1). Calculate the amount of each analyte in the sample by the following formula:

$$\text{The amount of each analyte in the sample (ppm)} = \frac{C \times V}{M} \times 10^{-3}$$

where,

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

V: the final make-up volume of the sample (20 mL)

M: the weight of sample (g)

GC-MS/MS operating conditions(note 2):

Column: HP-5MS UI capillary column, 0.25 μm, 0.25 mm i.d.× 30 m.

Column temperature:

Initial temperature: 50°C, 1 min;

Temperature rising rate: 25°C/min;

Middle temperature 1: 150°C, 1 min;

Temperature rising rate: 25°C/min;

Middle temperature 2: 200°C, 2 min;

Temperature rising rate: 30°C/min;

Final temperature: 290°C, 2 min.

Carrier gas and flow rate: helium, 2 mL/min.

Injection volume: 2 μL.

Injector temperature: 300°C.

Ion source temperature: 300°C.

Ionization mode: EI, 70 eV.

Injection mode: splitless.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair and collision energy are shown as follows.

Analyte	Ion pair Precursor ion (*m/z*) > product ion (*m/z*)	Collision energy (eV)
Imperatorin	202 > 89*	45
	174 > 89	40
5-Methoxypsoralen	216 > 89*	45
	173 > 89	20
8-Methoxypsoralen	216 > 89*	45
	173 > 89	20
6-Methylcoumarin	160 > 132*	10
	132 > 77	40
Musk ambrette	268 > 253*	5
	253 > 91	30
Safrole	162 > 104*	12
	162 > 131	12
Trioxysalen	228 > 199*	30
	199 > 128	30

*Quantitative ion pair

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 are as follows.

Relative ion intensity (%)	Tolerance (%)
> 50	± 10
> 20 - 50	± 15
> 10 - 20	± 20
≤ 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 quantification (LOQs) for 5-methoxypsoralen, 8-methoxypsoralen, 6-methylcoumarin, safrole and trioxysalen are 0.1 ppm, imperatorin and musk ambrette are 0.2 ppm.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Taiwan Food and Drug Administration. 2016. Method of test for safrole in soft drinks. (MOHWA0022.00). Published, Jun 3, 2016.
2. Viola, H., Frauke, D., Athanasios, N., Albrecht, F. and Norbert, A. 2017. A method for the quantification of 8-methoxypsoralen by mass spectrometry for offline extracorporeal photopheresis. Photochem. Photobiol. Sci. 16: 193-200.

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Reference chromatogram

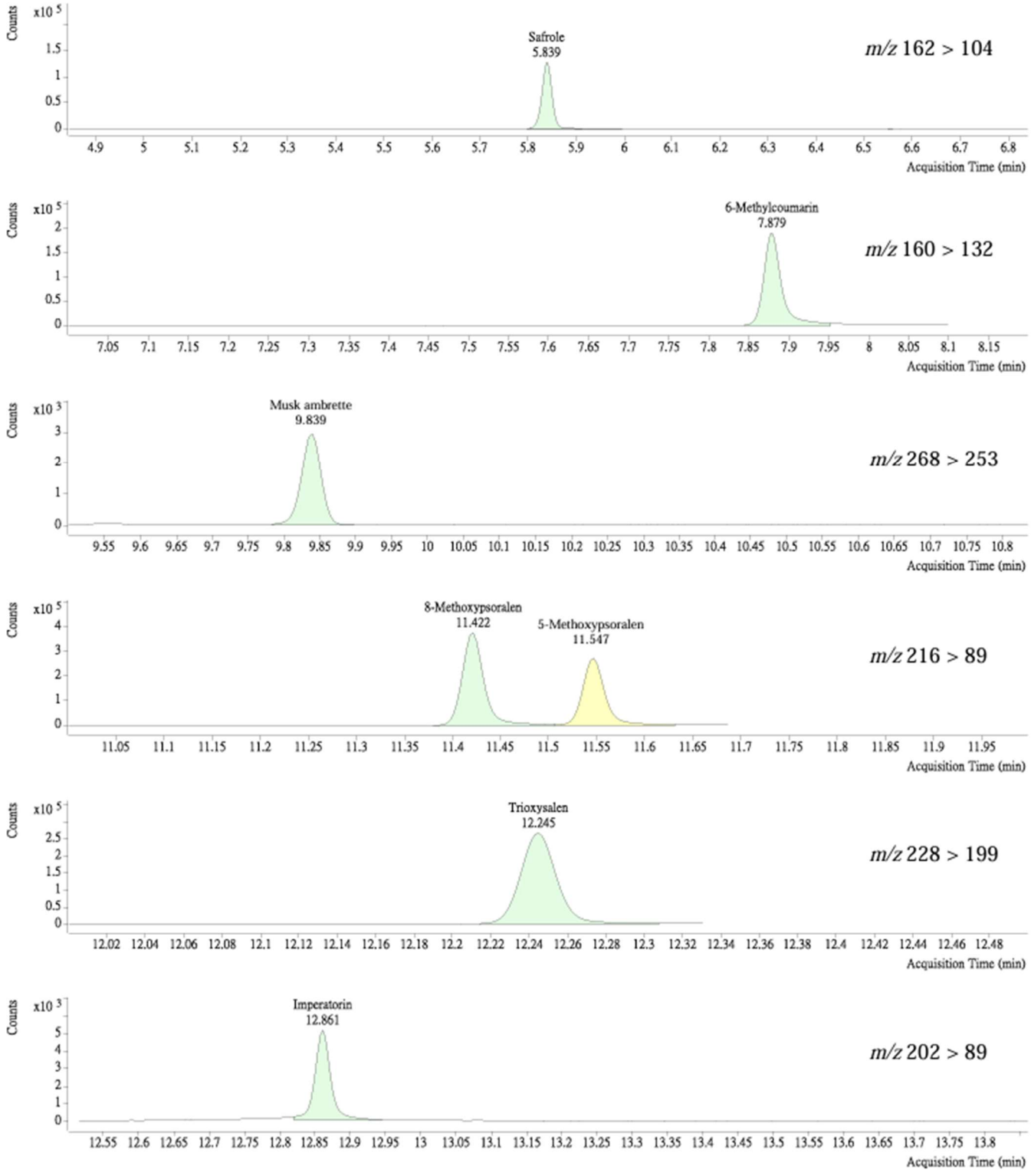

Figure. MRM chromatograms of 7 prohibited substance standards analyzed by GC-MS/MS.