

Japan Customs Analysis Methods

No. 302

Analysis Method of Peppermint Oil

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1. Scope

This analysis method is applied to peppermint oils of subheadings 3301.24 and 3301.25 of Customs Tariff Law (Appendix Table – Customs Tariff Schedule), for distinguishing between those obtained from *Mentha piperita* and those from *M. arvensis*.

2. Outline of Test Method

Under Customs Tariff Law (Appendix Table – Customs Tariff Schedule), peppermint oils are divided into three groups, i.e. those obtained from *M. piperita*, those obtained from *M. arvensis* and others. This analysis method enables to distinguish between peppermint oil obtained from *M. piperita* and that from *M. arvensis*.

The peppermint oils from these two *Mentha* species are distinguished using gas chromatograph mass spectrometer, by detecting menthofuran, sabinene hydrate and viridiflorol, all of which are specifically contained in those obtained from *M. piperita*, and comparing the content ratio of cineole and limonene commonly contained in these two types of oils.

3. Apparatus

(1) Gas chromatograph-mass spectrometer (GC-MS)

(2) Capillary column

Use a DB-WAX (30 m × 0.25 mm i.d., film thickness 0.5 µm) or equivalent one

(3) GC-MS, operating under the following conditions:

(a) GC temperature program

Program the oven temperature so that each constituents of peppermint oils are separated

sufficiently (e.g., initial temperature 50 °C for 3 min; increase at 15 °C per min; 110 °C; increase at 3 °C per min; 150 °C; increase at 15 °C per min; final temperature 200 °C for 5 min).

(b) Injection port temperature - 250 °C

(c) Others

Set other instrument parameters to the optimum conditions for the analysis.

4. Reagents

(a) Standard peppermint oil⁽¹⁾

Use peppermint oils obtained from *M. piperita* and *M. arvensis* whose constituents being main peaks in their gas chromatograms have been identified.

(b) Diethyl ether

JIS special reagent grade or equivalent

Note 1) Standard peppermint oils have to be stored in a cool and dark place.

5. Procedure

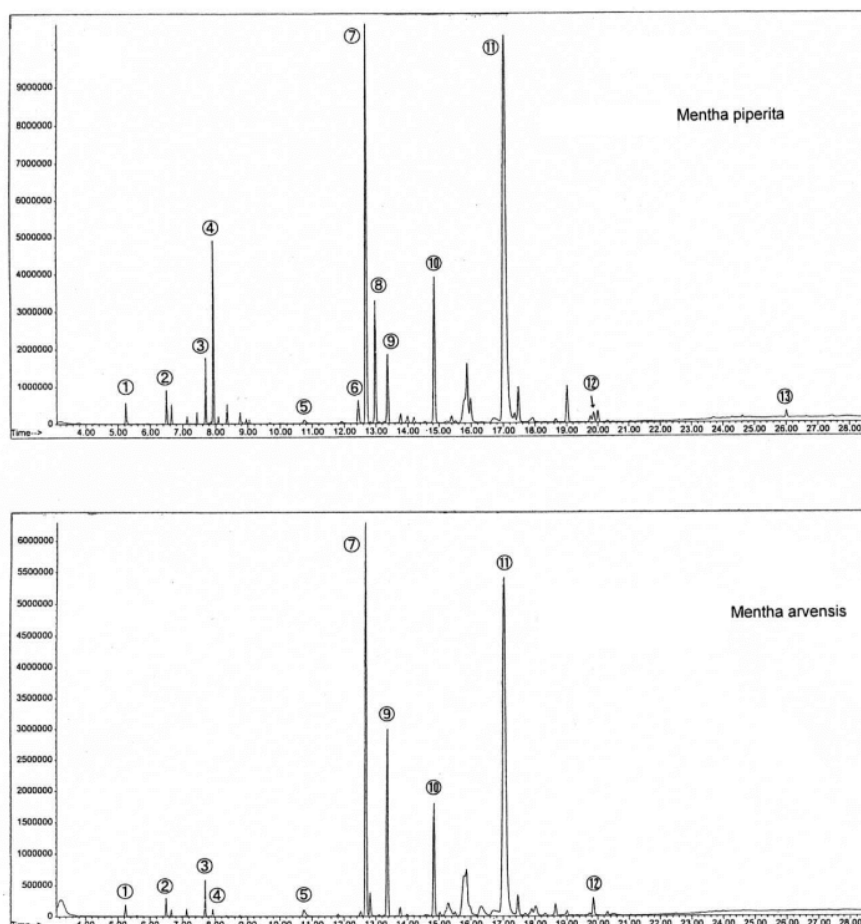
5.1. Measurement

Prepare a test solution of approx. 1 % concentration by adding diethyl ether to the test sample. Inject 1 µl of the test solution into GC-MS to obtain a total ion chromatogram and to measure mass spectra of object peaks.

5.2. Identification of constituents

Identify object peaks in the total ion chromatogram of the test sample, based on their mass spectra and by comparing their retention times with those of the same compounds present in the standard peppermint oils. ⁽²⁾

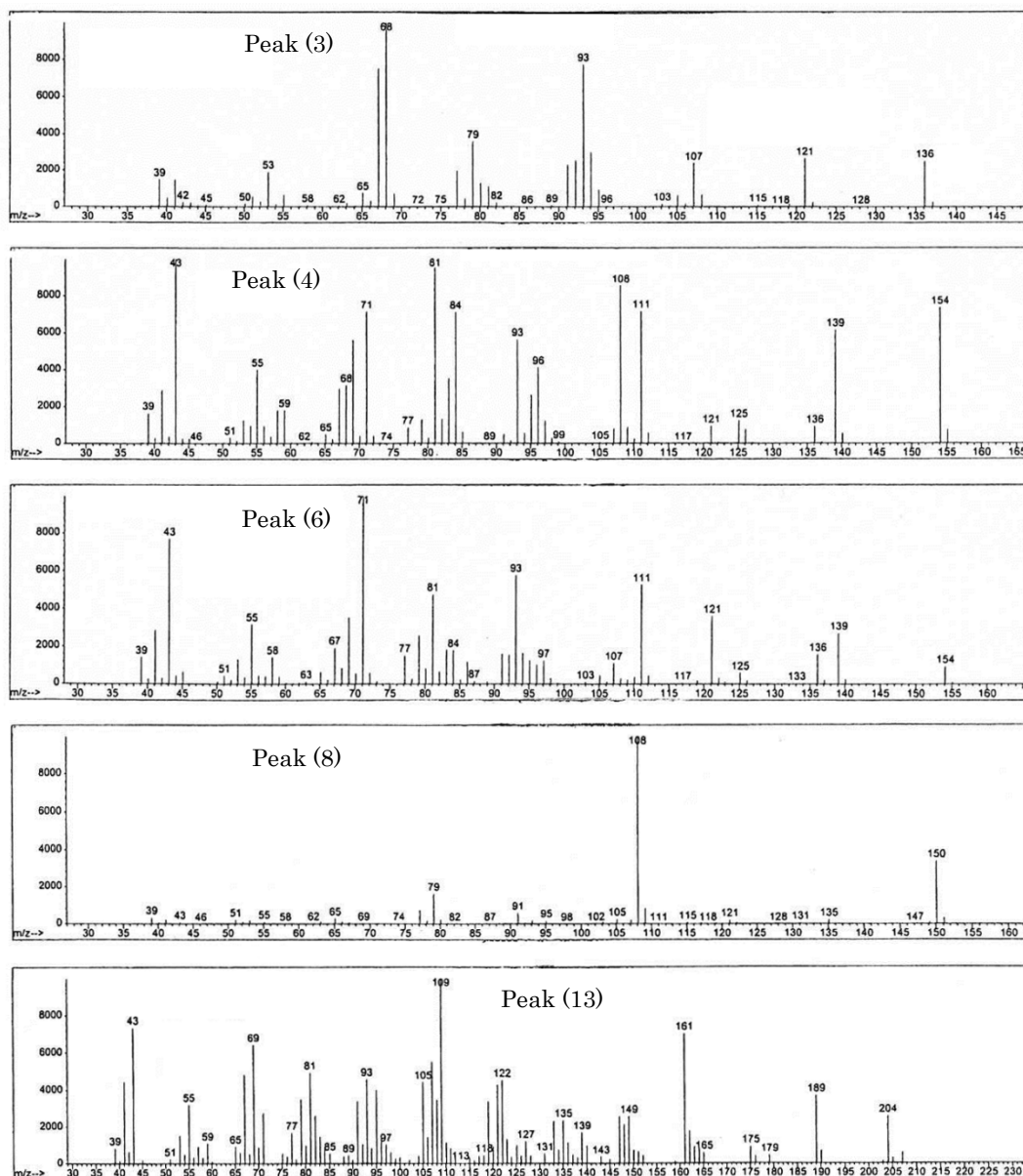
Note 2) When additional peaks other than peaks in the total ion chromatograms of the standard peppermint oils are found, the presence of additives in the test sample should be confirmed.



Reference 1: Examples of total ion chromatograms

Measurement conditions: Column, DB-WAX (30 m × 0.25 mm i.d., film thickness of 0.5 μm); Oven temperature, initial 50 °C (3 min.) → 15 °C/min → 110 °C → 3 °C/min → 150 °C → 15 °C/min → final 200 °C (5min); Carrier gas, Helium (1 ml/min); Injected volume, 1 μl; Injection port temperature, 250 °C; Injection port mode, split (20:1); Interface temperature, 250 °C; Ionization, EI, 70 eV.

Peak No.	Compound	Peak No.	Compound	Peak No.	Compound
1	α-Pinene	6	(+) trans-Sabinene hydrate	11	l-Menthol
2	β-Pinene	7	Menthone	12	Piperitone
3	d-Limonene	8	Menthofuran	13	Viridiflorol
4	Cineole	9	iso-Menthone		
5	3-Octanol	10	Menthylacetate		



Reference 2: Mass spectra of the main peaks

5.3. Identification of peppermint oils

Identify the kind of peppermint oil sample using the table below indicating differences between peppermint oil obtained from *M. piperita* and that obtained from *M. arvensis*.

If the test samples are mixtures of the two kinds or

obtained from hybrids between the two species, it is difficult to identify them using the criteria of the table below. In that case, comprehensive analysis is required, including quantitative studies on their physiochemical properties (specific gravity, refractive index, optical rotation and the like) and chemical compositions.

Table Criteria for discrimination between two kinds of peppermint oils

Item	Kind of peppermint oil	
	<i>M. piperita</i>	<i>M. arvensis</i>
Detection of menthofuran	(+)	(-) ⁽³⁾
Detection of sabinene hydrate	(+)	(-) ⁽³⁾
Detection of viridiflorol	(+)	(-) ⁽³⁾
Peak height of limonene and cineol	limonene < cineol	limonene > cineol

Note 3: Where, "(-)" indicates that the compound concerned is undetectable at a standard sensitivity level (which allows detection of the compound from standard *M. piperita* but not from *M. arvensis*).

5.4. Determination of Total Menthol

If the sample is identified to be peppermint oil obtained from *M. arvensis* according to the procedure in 5.3, its total content of menthol is determined by the following quantitative method as test method based on Article 74 of the Cabinet Order for Enforcement of the Customs Tariff Law.

Weigh about 10 g of the test sample into an extraction flask. Add 10 ml of acetic anhydride and 2 g of sodium acetate, anhydrous to the flask. Attach a reflux condenser to the flask and boil for 1 hour. Cool it and add 30 ml of water. Heat the flask in boiling water while stirring occasionally for 15 min.

Transfer the contents to a separating funnel to separate the oil layer. Wash the oil layer with water

until it becomes neutral. Add 2 g of sodium sulfate, anhydrous, and shake it vigorously. After leaving to stand for 30 min, filter it through a dried paper filter.

Accurately weigh about 2 g of the obtained (acetylated) oil and about 5 g of the test sample (unacetylated oil) into separate extraction flasks. Add 5 ml of ethanol and two drops of phenolphthalein solution⁽⁴⁾ to both flasks. After neutralizing both solutions by adding 0.5 mol/l of potassium hydroxide-ethanol solution, add 25 ml of 0.5 mol/l potassium hydroxide-ethanol solution. Attach a reflux condenser to the flask and boil the content for 1 hour. Add 25 ml of water immediately after boiling and stir. Cool the solution and titrate the excess amount of potassium hydrate with 0.5 mol/l of hydrochloric acid. Perform a blank test in the same manner.

$$total\ menthol, \% = \frac{7.814 \times (c - a) \left\{ 1 - \frac{0.021 \times (c - b)}{[unacetylated\ oil\ (g)]} \right\}}{[acetylated\ oil\ (g)] - 0.021 \times (c - a)}$$

Where- "a" and "b" represent the titration volumes (ml) of 0.5 mol/l of hydrochloric acid added to acetylated oil and unacetylated oil, respectively. "c" represents the titration volume (ml) of hydrochloric acid used in the blank test.

Note 4: Phenolphthalein solution is prepared by dissolving 1 g of phenolphthalein in 100 ml of ethanol.

6. References

- (1) Tada et al., (1971) Reports of the Central Customs Laboratory, **5**, 59-64
- (2) Formacek and Kubeczka: Essential Oil Analysis, John Wiley & Sons
- (3) The Japanese Pharmacopoeia, 11th Edition (Explanatory note)
- (4) 梅本和泰：ハッカ属植物の自殖株の精油成分，晃洋書房 (Japanese only)