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Application News

No.G285

Gas Chromatography

Analysis of Fatty Acid Content Ratios in Polysorbate 80

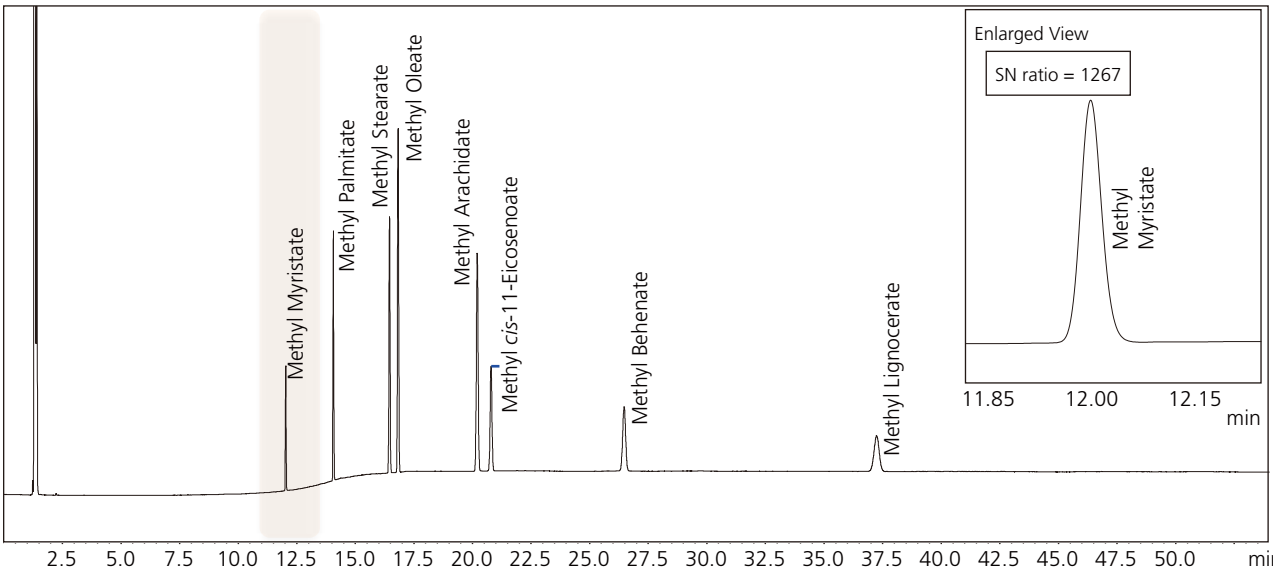
Polysorbate 80 is a highly safe water soluble emulsifier that, in addition to its use in ointments (creams) as an emulsifier, is also used as an injectable solubilizing agent for oil-soluble vitamins as well as in health drinks. There was a partial revision of the Japanese Pharmacopoeia (2011 Japanese Ministry of Health, Labour and Welfare Notification No. 65) as reported by the Japanese Ministry of Health, Labour and Welfare in its Notification No. 47 (February 28, 2014), and which became effective that same day. In this Pharmacopoeia Supplement, the section "Composition of fatty acids" was added to the Polysorbate 80 article in the official monographs.
A system suitability test has been established for this test method, and the system must conform to the test requirements.
Regarding the actual sample analysis, the product is to be dissolved in sodium hydroxide, and when conducting gas chromatographic analysis of the sample solution derivatized with boron trifluoride-methanol reagent, the required fatty acid content ratios have been specified as: myristic acid 5.0 % or less, palmitic acid 16.0 % or less, palmitoleic acid 8.0 % or less, stearic acid 6.0 % or less, oleic acid 58.0 % or less, linoleic acid 18.0 % or less, and linolenic acid 4.0 % or less. This Application News introduces the system suitability test associated with the analysis of fatty acid content ratios specified in Polysorbate 80 in the official monographs.

System Suitability Test

The test for required detectability is specified as follows: "Dissolve 0.50 g of the mixture of fatty acid methyl esters described in Table 1 in heptane to make 50.0 mL, and use this solution as the solution for the system suitability test. To 1.0 mL of the solution for the system suitability test, add heptane to bring the volume to 10.0 mL. When the procedure is run with 1 µL of this solution under the conditions of Table 2, the SN ratio of methyl myristate is to be not less than 5." The chromatogram obtained using a 1 µL injection of this solution is shown in Fig. 1. The SN ratio of methyl myristate is greater than 5.

Table 1 System Suitability Test Solution

Fatty Acids Methyl Ester Mixture	Content (%)
Methyl Myristate	5 %
Methyl Palmitate	10 %
Methyl Stearate	15 %
Methyl Arachidate	20 %
Methyl Oleate	20 %
Methyl *cis*-11-Eicosenoate	10 %
Methyl Behenate	10 %
Methyl Lignocerate	10 %



Note: The SN ratio is a reference value, and is not a guaranteed value.

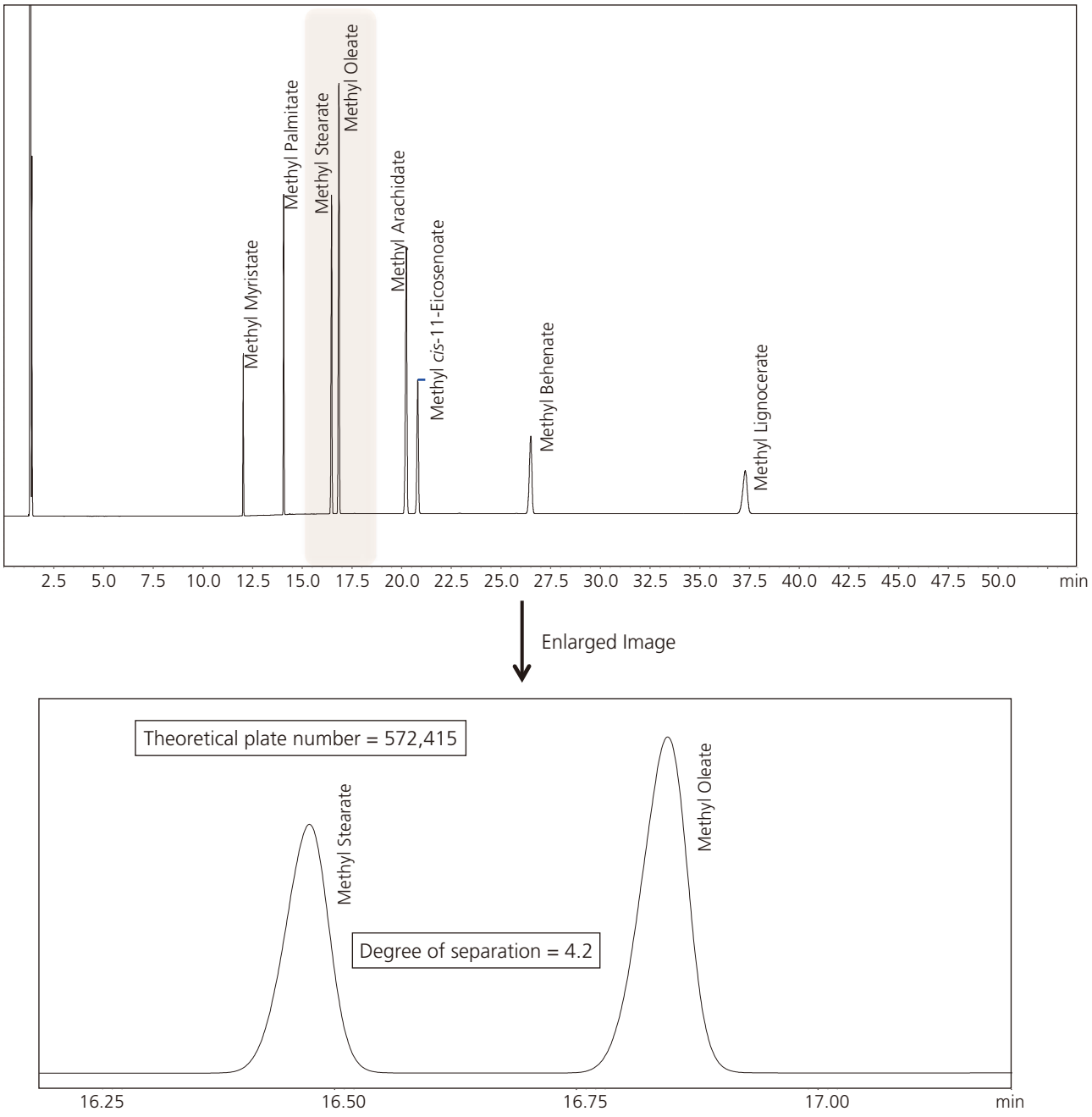
Fig. 1 Chromatogram of Tenfold Diluted System Suitability Test Solution

Table 2 Analytical Conditions

Model	: GC-2010 Plus AF, AOC-20i	Inj. Temp.	: 250 °C
Column	: Stabilwax (30 m × 0.32 mm I.D. df = 0.5 µm)	Det. Temp.	: 250 °C
Column Temp.	: 80 °C - 10 °C/min – 220 °C (40 min)	Split Ratio	: 1:20
Carrier Gas	: He, 50 cm/sec	Inj. Volume	: 1 µL

The system performance test is specified as follows: "When the procedure is run with 1 µL of the solution for the system suitability test under the conditions of Table 2, methyl stearate and methyl oleate are eluted in this order, the resolution between these peaks is not less than 1.8, and the number of theoretical plates of the peak of methyl stearate is not less than 30,000."

The chromatogram obtained using a 1 µL injection of this solution is shown in Fig. 2. The degree of separation of methyl stearate and methyl oleate is greater than 1.8, and the theoretical plate number of the methyl stearate peak is greater than 30,000.



Note: Degree of separation and theoretical plate number are reference values, and are not guaranteed values.

Fig. 2 Chromatogram of System Suitability Test Solution

[References]
Ministry of Health, Labor and Welfare Notification No. 47 (February 28, 2014)

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