GT086

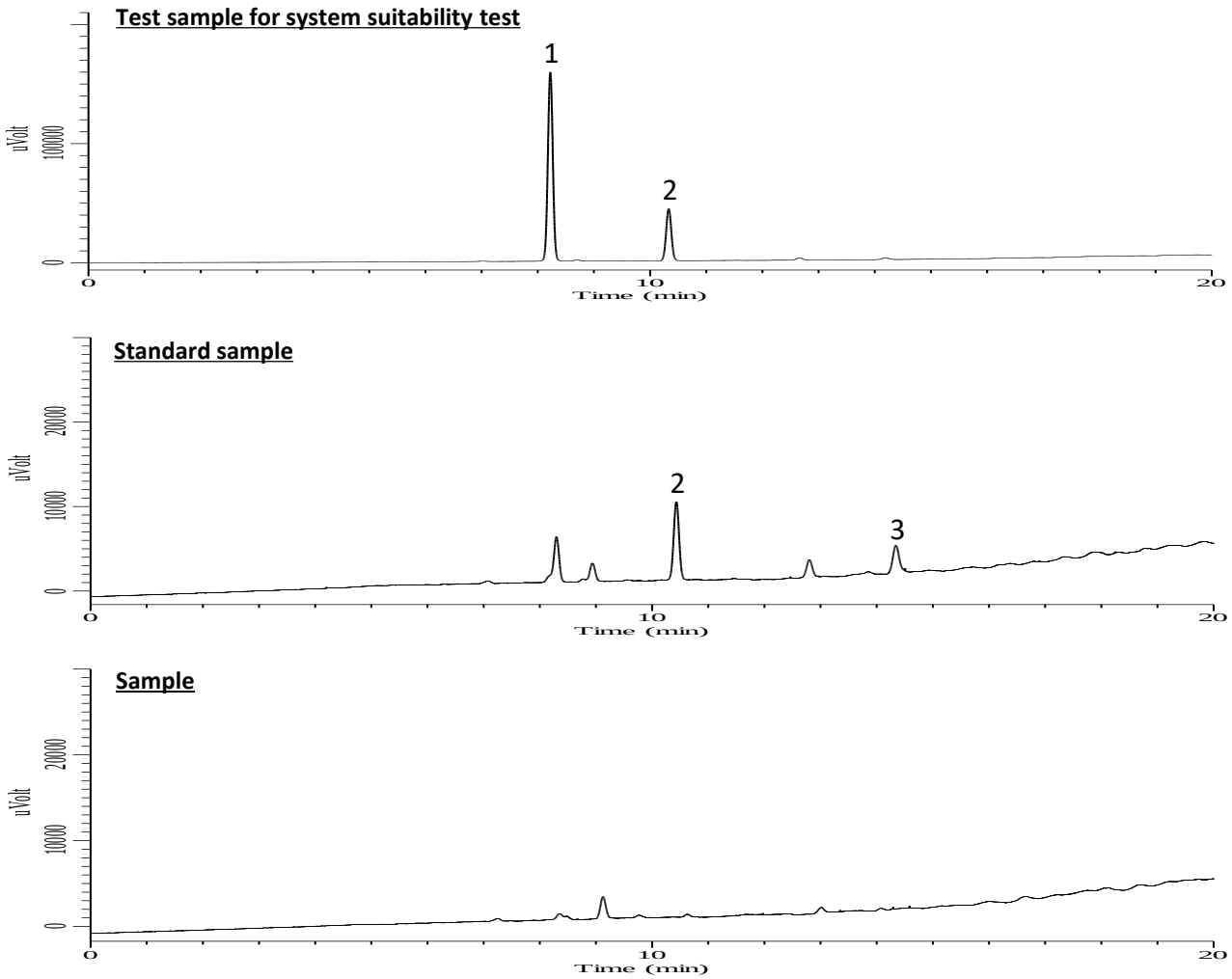
GL Sciences Inc.

Purity Test and Fatty Acid Content Ratio Test of Polysorbate 80 - Supplement II to the Japanese Pharmacopoeia, 16th Edition

A harmonized test was agreed for Polysorbate 80 in the three pharmacopoeias of Japan, Europe and the United States. Based on this agreement, test methods were revised in Supplement 2 of the Japanese Pharmacopoeia (JP16), 16th Edition, and analysis using gas chromatography (GC) was added.

In accordance with Supplement 2 of the Japanese Pharmacopoeia, 16th Edition, a purity test and fatty acid content ratio test were conducted. Excellent results were obtained.

Purity (2) Ethylene oxide and 1,4-dioxane



Conditions

System	: GC - FID - HS (Tekmar HT-3)
Column	: InertCap 5 0.53 mm I.D. x 50 m df = 5 µm (Cat.No. 1010-18459)
Col. Temp.	: 70 ˚C - 10 ˚C/min – 250 ˚C (5 min)
Carrier Gas	: He 30 kPa
Injection	: Split 3.5:1 85 ˚C
Injection Vol.	: Headspace Gas 1 mL
Detection	: FID Range 10^0 250 ˚C
Sample	: Standard
Sample heating temp.	: 80 ˚C Sample heating time 30 min
Headspace Vial	: 9.0 mL

Analyte

1. Acetaldehyde
2. Ethylene oxide
3. 1,4-Dioxane

Resolution (1, 2) : 12.77 [≧ 2.0]

* JP specified conditions in []

Test for fatty acid content ratio (system suitability)

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 %)

In the "Polysorbate 80" fatty acid content ratio test, it is necessary to prepare a mixed reagent of eight fatty acid methyl ester components. However, the concentration specified by the test method varies for each component; the preparation of this standard takes time and labor.
To simplify the preparation of the standard, the following is recommended; "Mixture of Fatty Acid Methyl Esters (Cat. No. 1021-58220)" diluted in n-heptane according to the test method and measured using a gas chromatograph – the mixing ratio is shown on the left.

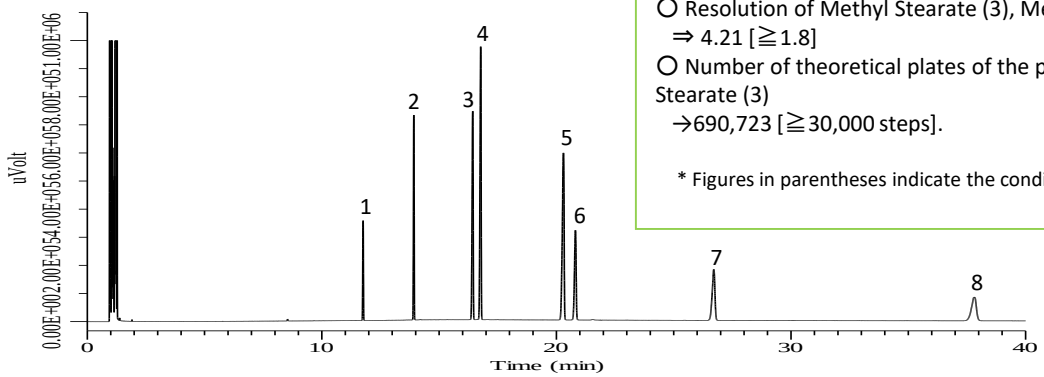
***One-point advice**

[If the weighing of the fatty acid methyl ester mixture is unsuccessful…]

The Fatty Acid Methyl Ester Mixture (Cat. No. 1021-58220) is supplied as a solid, it may be difficult to weigh in the solid state, If this occurs, warm it in a warm water bath and return it to a liquid state before use.
See page 4 of this Technical Note for details and prices, etc.

* A single-component sample of each component is also available. (undiluted)

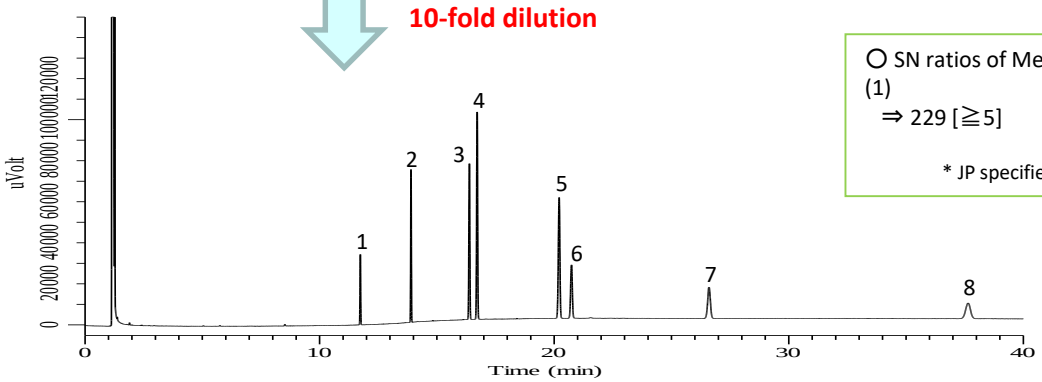
[Test sample for system suitability test]



○ Resolution of Methyl Stearate (3), Methyl Oleate (4)
⇒ 4.21 [≧1.8]
○ Number of theoretical plates of the peak of Methyl Stearate (3)
→690,723 [≧30,000 steps].

* Figures in parentheses indicate the conditions specified in the JP.

10-fold dilution



○ SN ratios of Methyl Myristate (1)
⇒ 229 [≧5]

* JP specified conditions in []

Conditions

System	: GC - FID
Column	: InertCap Pure-WAX 0.32 mm I.D. x 30 m df = 0.5 µm
Column Cat.No.	: 1010-68244
Col. Temp.	: 80 ˚C - 10 ˚C/min – 220 ˚C (40 min)
Carrier Gas	: He 110 kPa
Injection	: Split 1:20 250 ˚C
Injection Vol.	: 1 µL
Detection	: FID Range 10^0 250 ˚C
Sample	: Standard Analyte in Heptane

Analyte

1. Methyl Myristate
2. Methyl Palmitate
3. Methyl Stearate
4. Methyl Oleate
5. Methyl Arachidate
6. Methyl cis-11-Eicosenoate
7. Methyl Behenate
8. Methyl Lignocerate

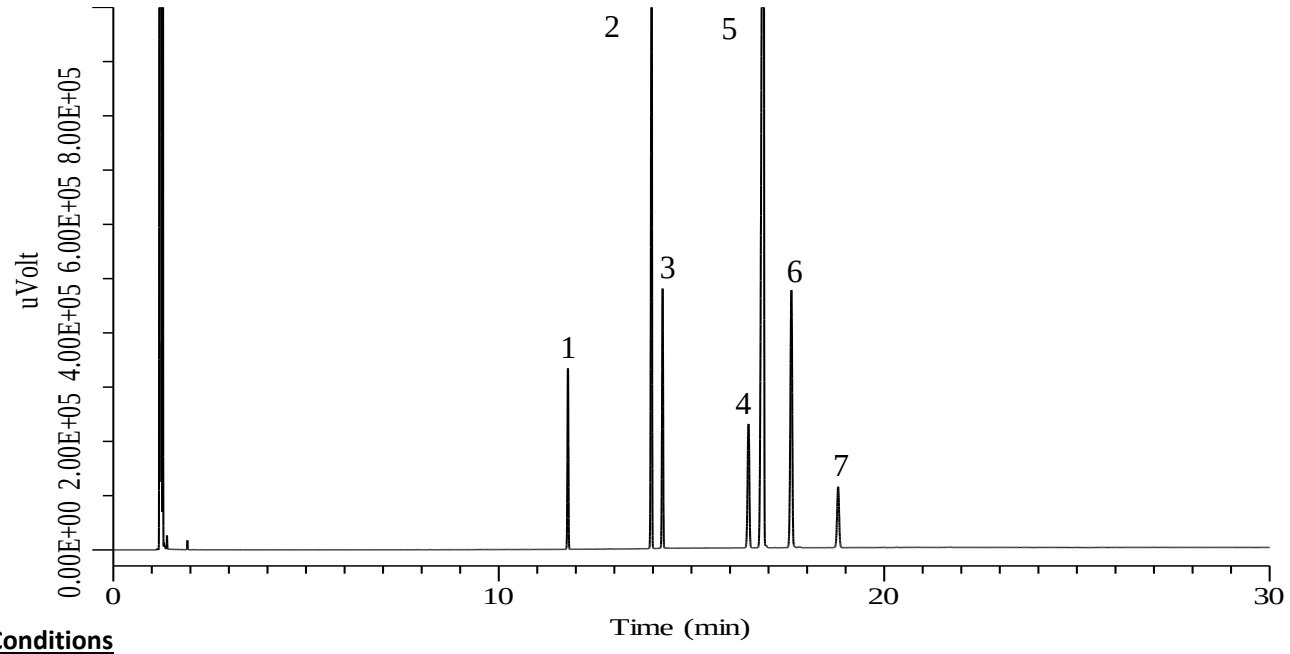
Test for fatty acid content ratio (reference: mixed fatty acid methyl ester TS)

Methyl Myristate (5%)
Methyl Palmitate (16%)
Methyl Palmitoleate (8%)
Methyl Stearate (6%)
Methyl Oleate (58%)
Methyl Linoleate (18%)
Methyl Linolenate (4%)

For the qualitative analysis of samples, a chromatogram of "mixed fatty acid methyl ester TS" should be used. This "mixed fatty acid methyl ester TS" should be prepared at the time of analysis because the type of fatty acid methyl ester is specified and there is no prior indication of the mixing ratio.

In this application, a sample with the mixing ratio shown on the left was diluted with n-heptane and measured using a gas chromatograph in accordance with the general test method for "Mixed fatty acid methyl ester TS."

※The mixing ratios listed on the left are with reference to the "Composition of fatty acids" of the European Pharmacopoeia (EP) and the "Acceptance Criteria" of the U.S. Pharmacopoeia (USP). In addition, the total mixing ratio was 115%, but in practice, the total mixing ratio was converted to 100% and the samples prepared as follows [Methyl Myristate (4%)、Methyl Palmitate (14%)、Methyl Palmitoleate (7%)、Methyl Stearate (5%)、Methyl Oleate (50%)、Methyl Linoleate (16%)、Methyl Linolenate (3%)]



Conditions

System	: GC-4000-FID
Column	: InertCap Pure-WAX 0.32 mm I.D. x 30 m df = 0.5 µm (Cat.No. 1010-68244)
Col. Temp.	: 80 ℃ - 10 ℃/min - 220℃ (40 min)
Carrier Gas	: He 110 kPa
Injection	: Split 1:20 250 ℃
Injection Vol.	: 1 µL
Detection	: FID Range 10^0 250 ℃
Sample	: Standard Analyte in Heptane

Analyte

1. Methyl Myristate
2. Methyl Palmitate
3. Methyl Palmitoleate
4. Methyl Stearate
5. Methyl Oleate
6. Methyl Linoleate
7. Methyl Linolenate

***One-point advice**

[If no desired reagent is found...]

Please consult GL Sciences
In addition to the procurement and arrangement of reagents, custom-made reagents are available on customer request.

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