nm, 320 nm, 326 nm, 330 nm, 340 nm and 350 nm, and calculate the relative extinction of each wavelength, taking the absorbance at 326 nm as 1.000.

When the wavelength of maximum absorption lies between 325 nm and 328 nm, and the relative extinction of each wavelength is within the range ±0.030 of the values in the Table, the potency of vitamin A in Unit per g of sample is calculated from the absorbance A at 326 nm.

$$\text{Units of vitamin A in 1 g} = E^{1\%}_{1\text{cm}}(326\text{ nm}) \times 1900$$

$$E^{1\%}_{1\text{cm}}(326\text{ nm}) = \frac{A}{W} \times \frac{V}{100}$$

V: Total mL of sample solution.
W: Amount (g) of sample in V mL of sample solution.

Perform the following test to identify retinol acetate and retinol palmitate.

Identification: Dissolve amounts or volumes, equivalent to 15,000 vitamin A Units, of the sample, Retinol Acetate Reference Standard for thin-layer chromatography and Retinol Palmitate Reference Standard for thin-layer chromatography in 5 mL portions of petroleum ether separately, and use these solutions as the sample solution and the standard solutions. Perform the test as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solutions on a plate of silica gel for thin-layer chromatography, develop it to a distance of about 10 cm with a mixture of cyclohexane and diethyl ether (12:1) as the solvent, and air-dry the plate. Spray antimony (III) chloride TS on the plate, and identify by comparing the position of the principal blue-colored spots of the sample and the standards.

When the wavelength of maximum absorption does not lie between 325 nm and 328 nm, or when the relative extinction is not within the range ±0.030 of values of the Table, proceed with the test by Method 2.

λ (nm)	Retinol acetate	Retinol palmitate
300	0.578	0.590
310	0.815	0.825
320	0.948	0.950
326	1.000	1.000
330	0.972	0.981
340	0.786	0.795
350	0.523	0.527

(2) Method 2

Unless otherwise specified, weigh accurately a sample containing not less than 500 Units of vitamin A, and not more than 1 g of fat, transfer to a flask, and add 30 mL of aldehyde-free ethanol and 1 mL of a solution of pyrogallol in ethanol (95) (1 in 10). Then add 3 mL of potassium hydroxide solution (9 in 10), attach a reflux condenser, and heat on a water bath for 30 minutes to saponify. Cool quickly to ordinary temperature, add 30 mL of water, transfer to separator A, wash the flask with 10 mL of water and 40 mL of diethyl ether, transfer the washings to separator A, shake well, and allow to stand. Transfer the water layer in separator A to separator B, wash the flask with 30 mL of diethyl ether, transfer the washing to separator B, and extract by shaking. Transfer the water layer to a flask, add the diethyl ether layer to separator A, transfer the water layer to separator B, add 30 mL of diethyl ether, and extract by shaking. Add the diethyl ether layer to separator A. Add 10 mL of water, allow to stand calmly after gentle turning upside down 2 to 3 times, and remove the water layer. Further wash with three 50-mL portions of water, and shake with increasing vigorousness as the washing proceeds. Further wash with 50-mL portions of water until the washing gives no pink color with phenolphthalein TS, and allow to stand for 10 minutes. Remove remaining water as far as possible, transfer the diethyl ether solution to an Erlenmeyer flask, wash the separator with two 10-mL portions of diethyl ether, add the washings to the flask, and add 5 g of anhydrous sodium sulfate to the diethyl ether solution, mix by shaking, and transfer the dried diethyl ether solution to a round-bottom flask by decantation. Wash the remaining sodium sulfate with two or more 10-mL portions of diethyl ether, and transfer the washings to the flask.

Evaporate the diethyl ether solution in a water bath at 45°C with swirling of the flask, using an aspirator, to about 1 mL, immediately add sufficient 2-propanol to make a solution containing 6 to 10 vitamin A Units per mL, and designate the solution as the sample solution. Read the absorbances, A_1 at 310 nm, A_2 at 325 nm, and A_3 at 334 nm with the sample solution as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Units of vitamin A in 1 g of sample} = E^{1\%}_{1\text{ cm}}(325\text{ nm}) \times 1830$$

$$E^{1\%}_{1\text{ cm}}(325\text{ nm}) = \frac{A_2}{W} \times \frac{V}{100} \times f$$

$$f = 6.815 - 2.555 \times \frac{A_1}{A_2} - 4.260 \times \frac{A_3}{A_2}$$

f: Correction factor
V: Total mL of sample solution
W: Amount (g) of sample in V mL of sample solution

67. Volatile Contaminants in Ethanol

The Volatile Contaminants in Ethanol is a method to determine the amount of methanol, acetaldehyde, acetone, 2-propanol, 1-propanol or *t*-butanol which are contained in ethanol using the Gas Chromatography.

Procedure

Take exactly 25 mL of the sample, add exactly 3 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh each accurately 5.0 g of methanol, 5.0 g of acetaldehyde for assay, 2.0 g of acetone, 1.0 g of 2-propanol, 1.3 g of 1-propanol and 1.3 g of *t*-butanol, and add ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL. To exactly 25 mL of this solution add 3 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions. Determine each peak area from both solutions by the automatic integration method, and calcu-

late the ratios, Q_{TA}, Q_{TB}, Q_{TC}, Q_{TD}, Q_{TE}, Q_{TF}, Q_{SA}, Q_{SB}, Q_{SC}, Q_{SD}, Q_{SE}, Q_{SF}, of the peak area of methanol, acetaldehyde, acetone, 2-propanol, 1-propanol and *t*-butanol to that of the internal standard. Q_{TA}, Q_{TB}, Q_{TC}, Q_{TD}, Q_{TE} and Q_{TF} are not larger than Q_{SA}, Q_{SB}, Q_{SC}, Q_{SD}, Q_{SE} and Q_{SF}, respectively.

Internal standard solution—A solution of tetrahydrofuran in ethanol (99.5) (1 in 500)

System operating conditions—

Detector: A hydrogen flame-ionization detector.

Packing: A globular porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (packed column)

Column temperature: A constant temperature of about 100°C.

Carrier gas: Nitrogen

System suitability test—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, methanol, acetaldehyde, ethanol, acetone, 2-propanol, 1-propanol, *t*-butanol and the internal standard are eluted in this order with well separation and with peak areas big enough to be determined. The peaks of the substances appear after ethanol may be shown on the tail of ethanol peak.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of methanol to that of the internal standard is not more than 10%.

68. Water Determination (Karl Fischer Method)

Water Determination is a method to determine water content in sample materials, utilizing the fact that water reacts with iodine and sulfur dioxide quantitatively in the presence of a lower alcohol such as methanol, and an organic base such as pyridine. The reaction proceeds in the manner shown in the following equation:

$$I_2 + SO_2 + 3C_5H_5N + CH_3OH + H_2O \rightarrow 2(C_5H_5N^+H)I^- + (C_5H_5N^+H)^-OSO_2OCH_3$$

In this measurement there are two methods different in iodine-providing principle: one is the volumetric titration method and the other, the coulometric titration method. In the former, iodine is previously dissolved in a reagent for water determination, and water content is determined by measuring the amount of iodine consumed as a result of reaction with water. In the latter, iodine is produced by electrolysis of Karl Fisher reagent containing iodide ion. Based on the quantitative reaction of the generated iodine with water, the water content in a sample specimen can be determined by measuring the quantity of electricity which is required for the production of iodine during the titration.

$$2I^- \rightarrow I_2 + 2e^-$$

1. Volumetric titration

Apparatus

Generally, the apparatus consists of automatic burettes, a titration flask, a stirrer, and equipment for amperometric titration at constant voltage or potentiometric titration at constant current.

The Karl Fischer TS is extremely hygroscopic, so the apparatus should be designed to be protected from atmospheric moisture. Desiccants such as silica gel or calcium chloride for water determination are used for moisture protection.

Reagents

(1) Chloroform for water determination—To 1000 mL of chloroform add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of chloroform. Preserve the chloroform, protecting it from moisture. The water content of this chloroform should not be more than 0.1 mg per mL.

(2) Methanol for water determination—To 1000 mL of methanol add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of methanol. Preserve the methanol, protecting it from moisture. The water content of this methanol should not be more than 0.1 mg per mL.

(3) Propylene carbonate for water determination—To 1000 mL of propylene carbonate add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear propylene carbonate layer. Preserve this protecting from moisture. The water content should not be more than 0.3 mg per mL.

(4) Diethylene glycol monoethyl ether for water determination—To 1000 mL of diethylene glycol monoethyl ether add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of diethylene glycol monoethyl ether. Preserve the diethylene glycol monoethyl ether, protecting it from moisture. The water content of this diethylene glycol monoethyl ether should not be more than 0.3 mg per mL.

(5) Pyridine for water determination—Add potassium hydroxide or barium oxide to pyridine, stopper tightly, and allow to stand for several days. Distill and preserve the purified and dried pyridine, protecting it from moisture. The water content of this pyridine should not be more than 1 mg per mL.

(6) Imidazole for water determination—Use imidazole for thin-layer chromatography, of which the water content should not be more than 1 mg per g.

(7) 2-Methylaminopyridine for water determination—Distill and preserve 2-methylaminopyridine, protecting it from moisture. The water content of this 2-methylaminopyridine should not be more than 1 mg per mL.

Preparation of test solutions and standard solutions

(1) Karl Fischer TS for water determination

Prepare according to the following method (i), (ii) or (iii).

(i) Preparation 1

Dissolve 63 g of iodine in 100 mL of pyridine for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 32 g. Then make up to 500 mL by adding chloroform for water determination or methanol for water determination, and allow to stand for more than 24 hours before use.