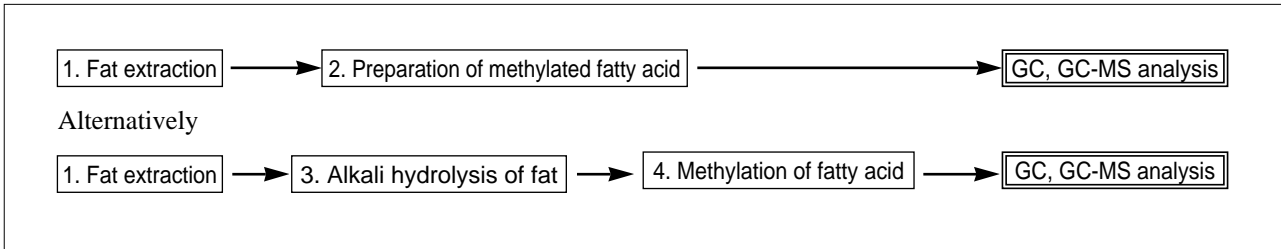




## 1.1 Analysis of Fatty Acids (3) / Derivatization - Fat Extraction Method

•Pretreatment for Fatty Acid Analysis  
Fat must be extracted from the food product and hydrolysis and methylation performed for GC and GC-MS analysis of fatty acids in food products. Here, several

representative pretreatment methods will be introduced from the numerous methods available.



### 1. Fat Extraction

This shows an example of fat extracted from a sample.

### References

Standard Methods of Analysis for Hygienic Chemists and Notes 1990 Appended supplement (1995)  
Pharmaceutical Society of Japan Edition, published by Kanehara & Co., Ltd (1995)

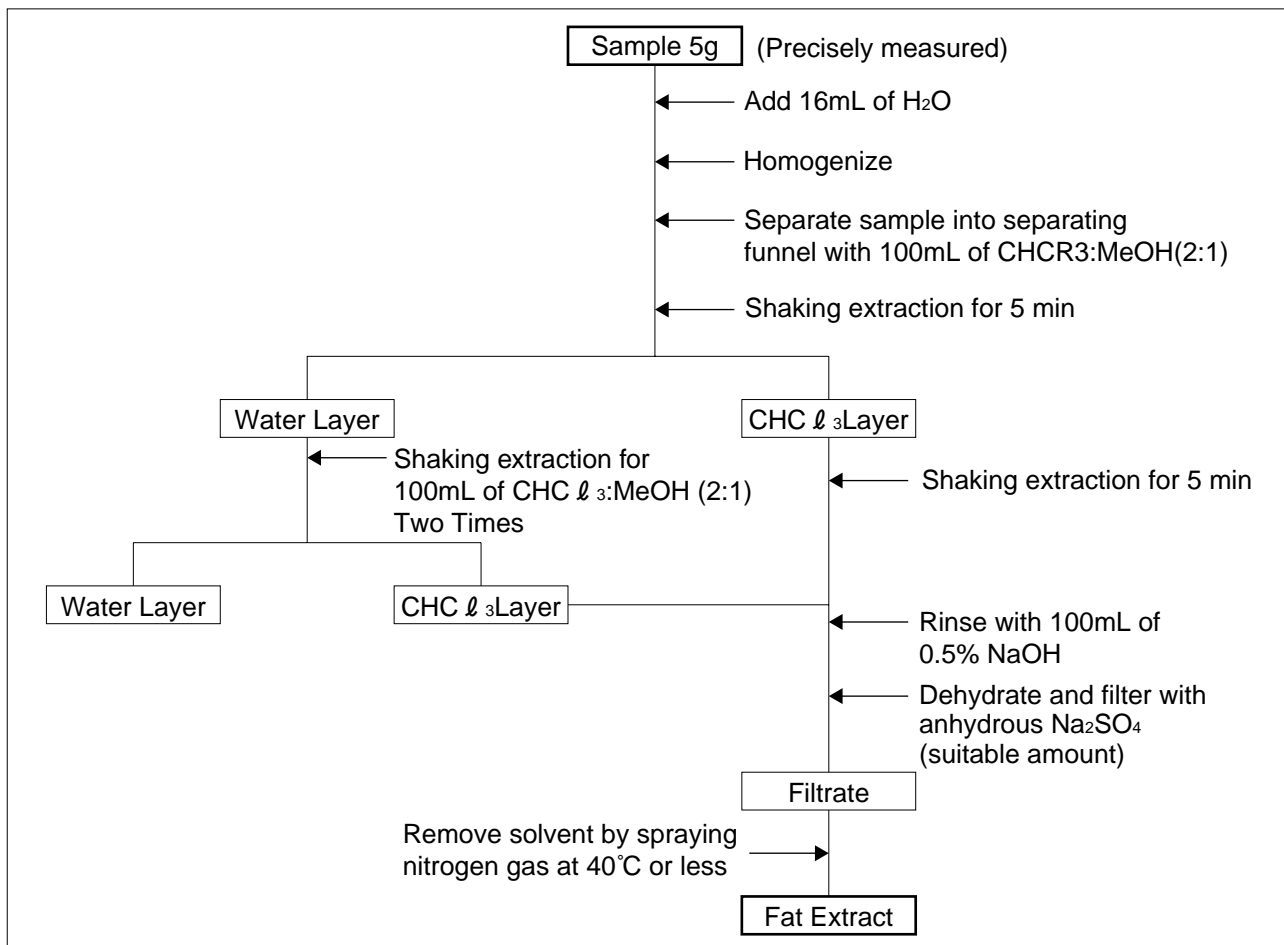


Fig. 1.1.6 Fat extraction method

## 1.1 Analysis of Fatty Acids (4) / Derivatization - Preparation of Methyl Fatty Acids

### 2. Preparation of Methylated Fatty Acid

This shows a transesterification method for extracting fat using an alkali catalyst that does not require fat extraction of food oils, etc. This easy method just requires hydrolysis and fatty acid extraction so labor is reduced.

Note, however, that amide-bonded fatty acid and free fatty acid do not methylate.

### References

Standard Methods of Analysis for Hygiene Chemists and Notes 1990 Appended supplement (1995)  
Pharmaceutical Society of Japan Edition, published by Kanehara & Co., Ltd (1995)

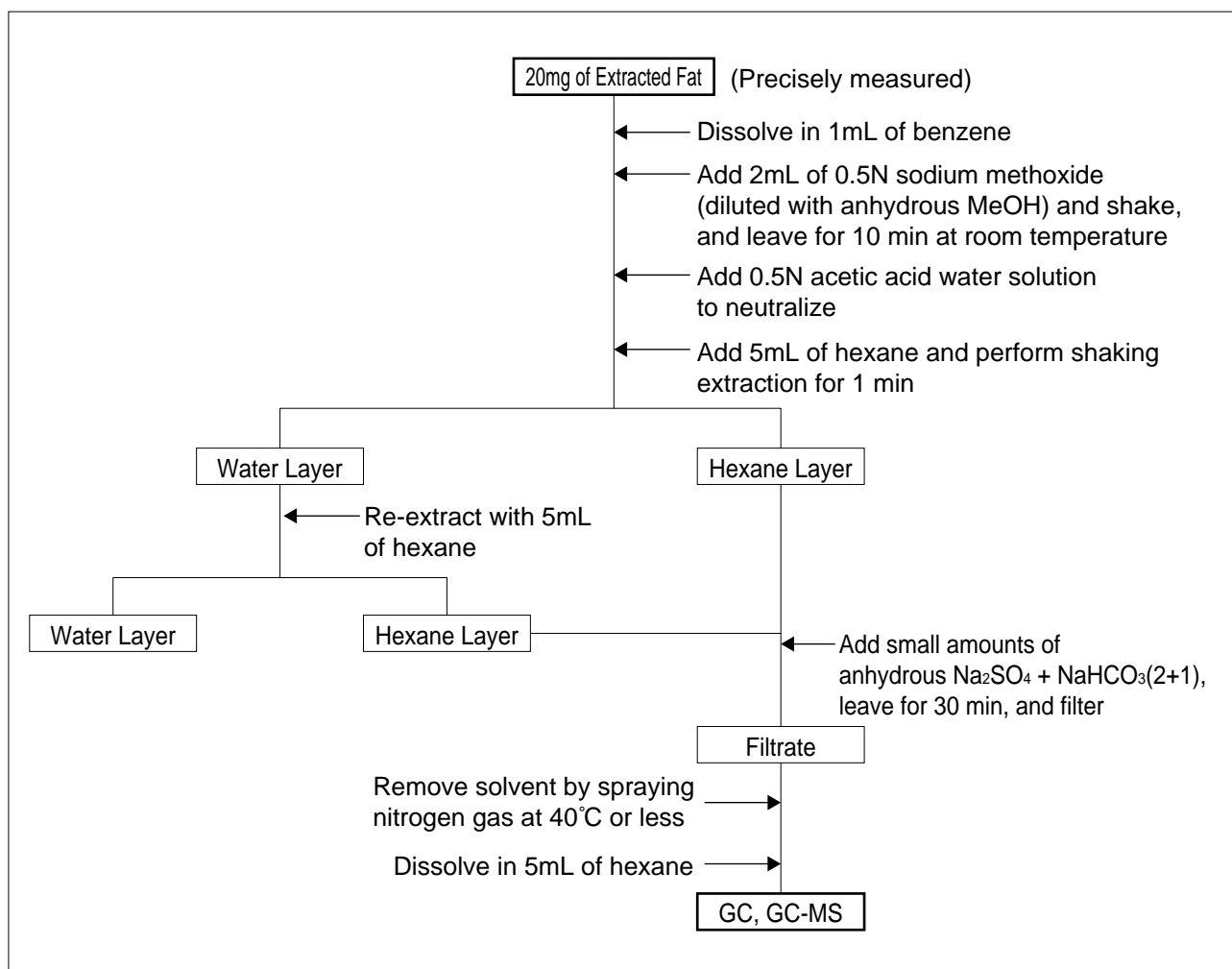


Fig. 1.1.7 Preparation of methylated fatty acid



## 1.1 Analysis of Fatty Acids (5) / Derivatization - Alkali Hydrolysis of Fat

### 3. Alkali Hydrolysis of Fat

Extracted fat is triacylglycerol which emerges as glycerol and potassium salt's fatty acid (water soluble) using alkali. Fatty acid hardly separates when acidified, which

enables extraction with non-polar solvent. Here, an example of alkali hydrolysis is introduced.

### References

Organic Chemistry Testing Guidebook No. 5, Handling Biological materials, Toshio Goto, Tetsuo Shiba, Teruo Matsuura ed, Kagaku-Dojin Publishing Company, INC (1991)

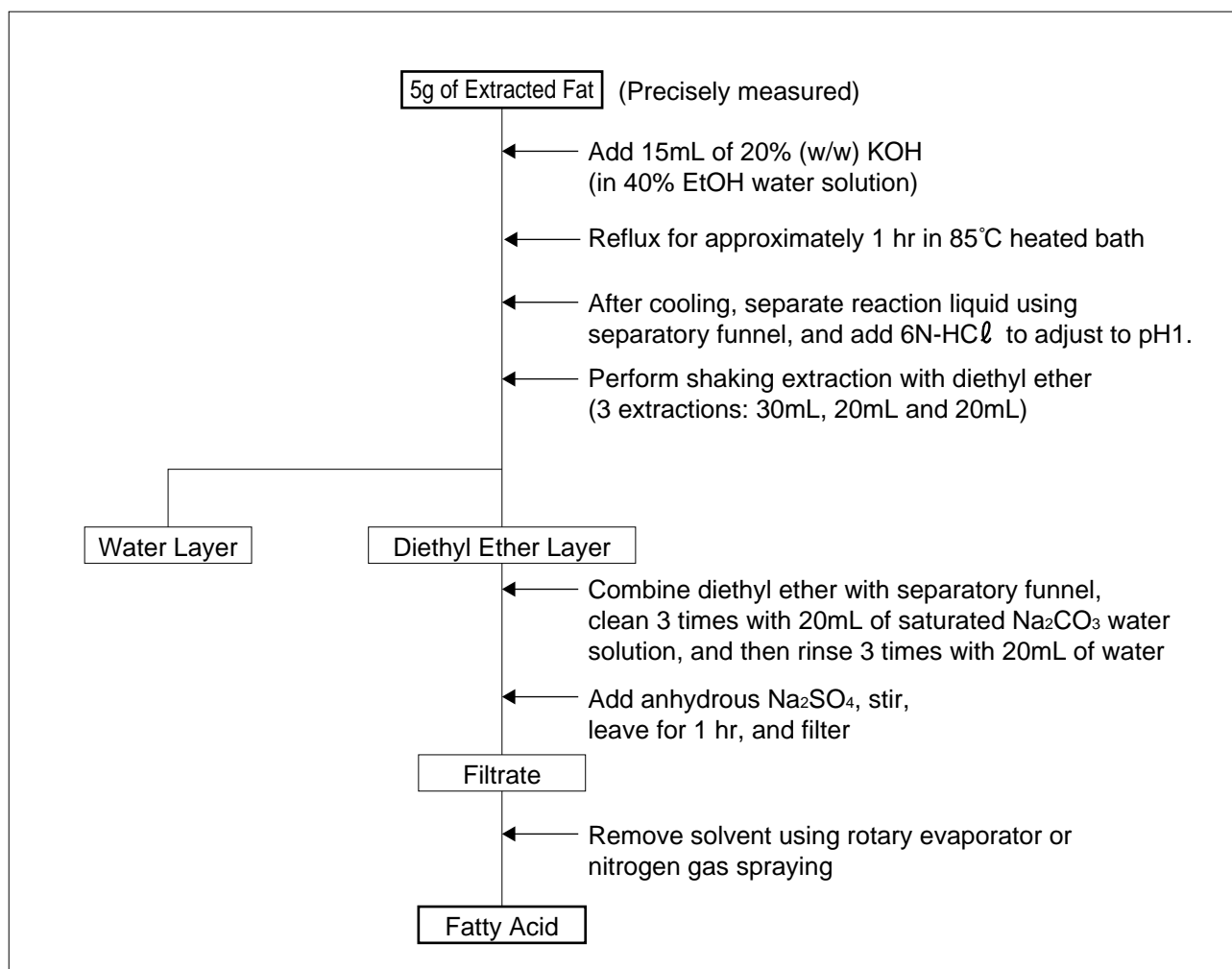


Fig. 1.1.8 Alkali hydrolysis of fat

## 1.1 Analysis of Fatty Acids (6) / Derivatization (1) - Preparation of Methyl Ester Derivative

### 4. Methyl Ester Derivative Preparation Method

High-class fatty acids are generally derived into methyl ester. The currently used methods are introduced here.

#### (1) Methyl Esterization using $\text{BF}_3\text{-CH}_3\text{OH}$

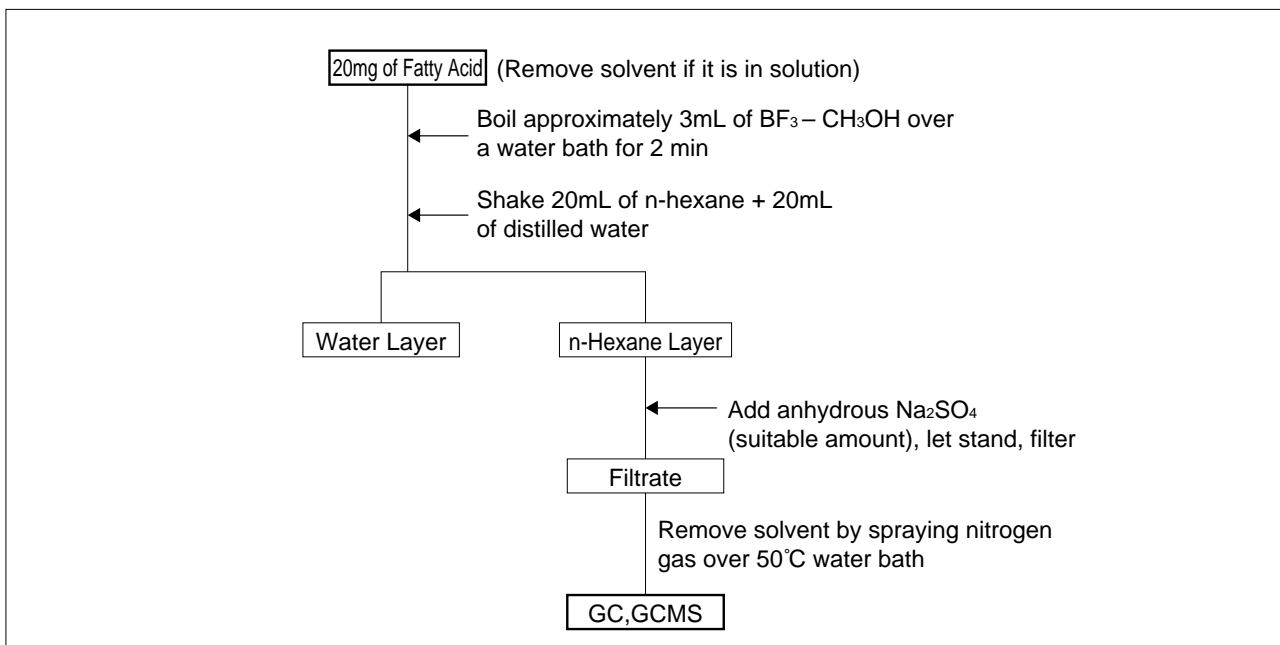


Fig. 1.1.9 Methyl esterization using boron trifluoride-methanol

#### (2) Methyl Esterization Using $\text{H}_2\text{SO}_4\text{-CH}_3\text{OH}$

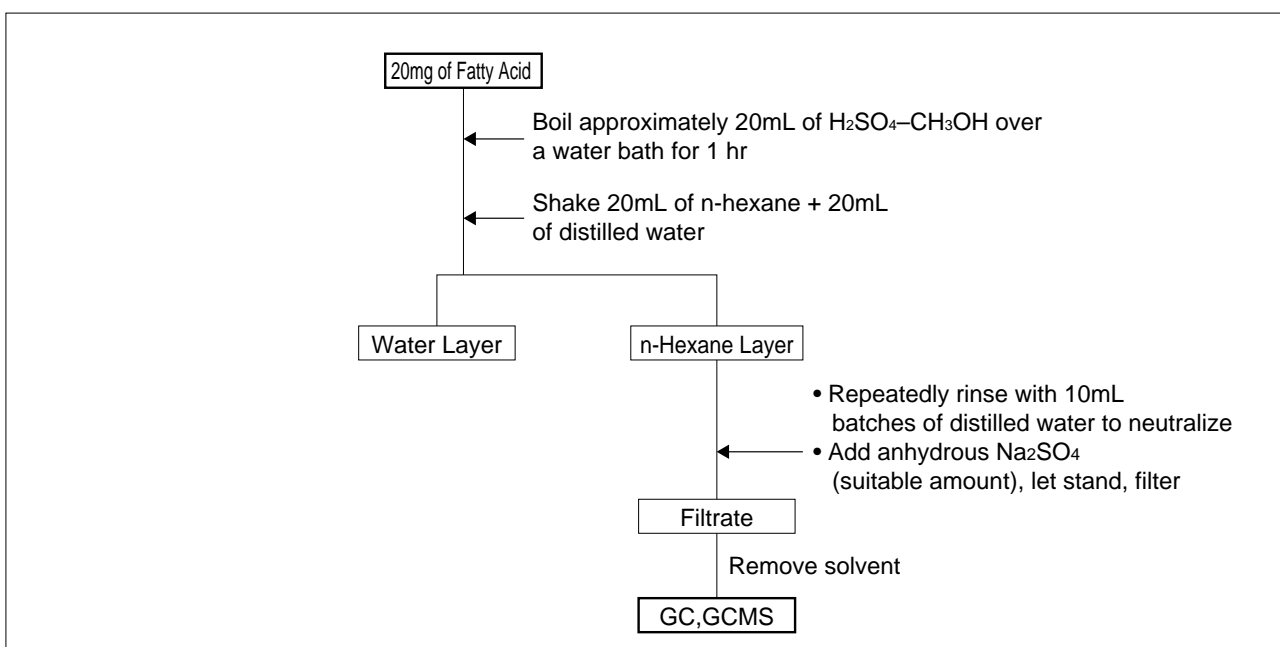


Fig. 1.1.10 Methyl esterization using sulphuric acid-methanol



## 1.1 Analysis of Fatty Acids (6) / Derivatization (2) - Methyl Ester Derivative

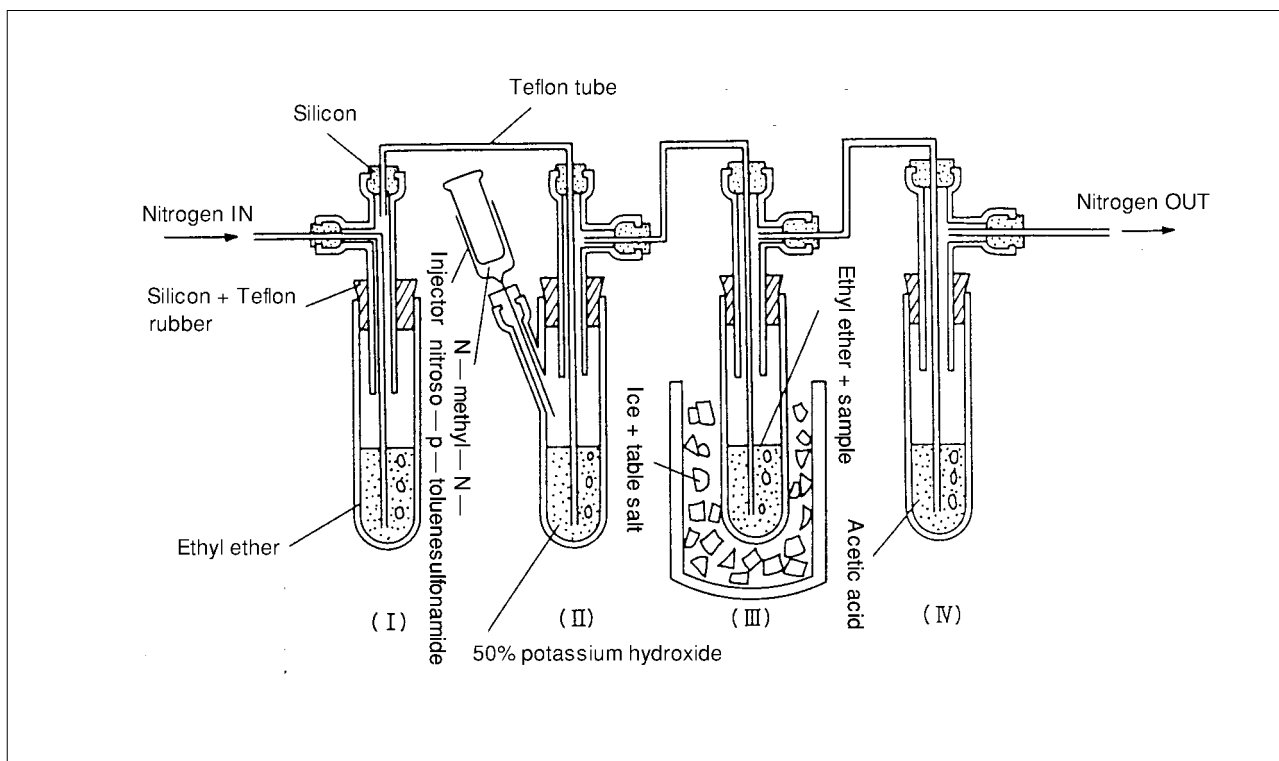


Fig. 1.1.11 Methyl esterization using diazomethane

### (3) Methyl Esterization Using $\text{CH}_2\text{N}_2$

A diazomethane generator is assembled as shown in the diagram. And ethyl ether (I), 50% potassium hydroxide water solution (II), 10mg of fatty acid + 2mL of ethyl ether (III) and acetic acid are sealed in tubes.

1. A suitable amount of nitrogen gas is passed through test tube I.
2. Some 0.5 to 1mL of N-methyl-N'-nitroso-p-toluenesulfonamide with 20% ethyl ether is injected into test tube II to create diazomethane.
3. Remove test tube III from diazomethane generator once the ethyl ether liquid inside has turned yellow.
4. Leave test tube III to stand for 10 min to enrich the ethyl ether, and inject into GC or GCMS.

#### ◆ Notes and cautions

- Handle diazomethane with care, as it is carcinogenic.
- For the above reason, only adjust small amounts and be sure to use a ventilating hood.
- Do not use ground glass stoppers because there is a danger of explosion.
- Small amounts of ether solution (100mL or less) can be stored in a refrigerator for several days.
- ◆ Several relatively easy-to-handle diazomethane generators are available in market.

### (4) Methyl Esterization Using Dimethylformamide Dialkylacetals $(\text{CH}_3)_2\text{NCH}(\text{OR})_2$

Add 300  $\mu\text{L}$  of esterification reagent to some 5 to 50mg of fatty acid. Dissolve the sample and inject the resultant reaction liquid into the GC or GCMS. (Normally it is best to heat this at 60°C for 10 to 15 min.)

### (5) Methyl Esterization Using Phenyltrimethyl Ammonium Hydroxide (PTAH)

Dissolve the fatty acid in acetone, add PTAH/methanol solution (1 to 1.5M%), thoroughly stir sample and reaction reagent, leave to stand for 30 min, and induct into GC or GCMS.

This methyl esterization using on-column injection is a method where the PTAH/methanol reagent and fatty acid are mixed in advance, injected into the GC and made to react in a GC injector. Compared to other methods treatment is quick and simple and there is no volatile loss because the reaction is in a GC injector. Furthermore, harmful, dangerous reagents are not required.