

## DEVELOPMENT OF SIMPLE ANALYTICAL METHOD OF OMEGA-3 FATTY ACID BY PROPANOIC ACID DETERMINATION USING ALKALIMETRIC TITRATION

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### ABSTRACT

The content of omega-3 in the fish oil capsules have been determined by alkalimetric titration method. This method has been done in three ways. Firstly, fatty acids were oxidized by  $KMnO_4$  using  $H_2SO_4$  as catalyst. Secondly, propanoic acid as the result of oxidation was separated by distillation. Furthermore, distilled propanoic acid was titrated. Precision, accuracy, repeatability, and reproducibility of the research were good enough for further development as a routine method for determination of omega-3 content.

**Keywords:** omega-3, alkalimetric titration.

### INTRODUCTION

Sophisticated knowledge in Biochemistry field has stated that the importance of fat for human body is depends on the component of fatty acids. There are two kinds of fatty acids: saturated fatty acid that contains double bond and unsaturated fatty acid which have no double bond. Generally, unsaturated fatty acid provides better effect for health than the saturated. One of important unsaturated fatty acid for human body is omega-3 fatty acid.

Because of its important role for health relating to brain work, body defence etc, it would be necessary to analyse the content of omega-3 fatty acids in our daily diet. So far, the common method for analysis the omega-3 fatty acid was using Gas Chromatography (GC) or Gas Chromatography-Mass Spectrometry (GC-MS) by transesterification process [1]. This method could be used to analyse the fatty acid content of any fat sample with good accuracy. Kristianingrum and Handayani have used this method to analyse the content of omega-3 fatty acid in snail meat[2]. Handayani analysed the fatty acid content in lard using the same method [3]. Although giving a good result, there is a lack of the method related to economical reason, i.e. expensive process including expensive instrument such as GC-MS, requiring expensive reagent and difficulties of interpretation and calculation data by the operator. Therefore, a simple, easy, fast and cheap method for omega-3 fatty acid analysis is necessary to developed.

Omega-3 fatty acid is a polyunsaturated fatty acid with double bond in last number 3 of carbon, so then called omega-3. The next double bonds are in the 3-carbon interval and conjugated with methylene ( $CH_2$ ) [4]. Alkenes can be oxidized in double bond to form a carboxylic acid and omega-3 fatty acid could be oxidized to form propanoic acid in the end of chain. For example, oxidation of linolenic acid produces propanoic and

dicarboxylic acids as shown in Fig 1. The most common oxidator for the alkenes oxidation is  $KMnO_4$  in acid condition. The resulted propanoic acid is a characteristic product of omega-3 oxidation. Propanoic acid has boiling point at  $141^\circ C$ , whereas the other products that contain two carboxylic acids has higher boiling point so it is easy to separate the propanoic acid [5]. Short chain fatty acid separation from the oil sample can be done with Reichert Meisel method, which is determined butyric and kaproic acid in oil or fat like butter or margarine [6].

The mol number of resulted propanoic acid is equivalent to the omega-3 fatty acid, which means that we can calculate omega-3 fatty acid concentration by determining the concentration of propanoic acid. Handayani has reported the preliminary research in determination of omega-3 fatty acid concentration using alkalimetric titration of propanoic acid as the product of fatty acid oxidation [7]. The method had some lacks because it still have 23.37% bias, 40.21% relative error and 10.26% limit detection. Therefore, this method needs to be improved by enhancing oxidation and distillation method, then testing repeatability and reproducibility of the developed method. This research will focus on calculating the repeatability and reproducibility of determination method of omega-3 content with the alkalimetric titration to propanoic acid as the product of omega-3 fatty acid oxidation. Fish oil sample was chosen as it shows the omega-3 composition in the label for comparing the yield.

### EXPERIMENTAL SECTION

#### Analysis Method

A mixture solution containing fish oil sample that dissolved in paraffin, sulphuric acid, potassium permanganate and aquades were refluxed with

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polysorbate as catalyst. After it was finish, the mixture distillate by Reichert Meisel method. The distillate was titrated with sodium hydroxide after it was standardized by oxalate acid.

#### Recovery factor determination

Recovery factor is necessary to determine in order to improve the method. Diluting 0.290 g propanoic acid in 100 mL aquades as standard solution did recovery factor. A mixture contains 10 mL standard, sulphuric acid and aquades then distilled with the same method for sample distillation. The yield was titrated by 0.1 M sodium hydroxide standard solution and pp as indicator (a). 10 mL propanoic acid standard was then titrated sodium hydroxide with pp indicator (b). Recovery factor is a/b.

#### GC-MS standard method as comparison

One gram sample weighted in closed tube then diluted in 2 mL hexane, added by 2 mL BF<sub>3</sub>/Methanol 14 %. The mixture solution was heated in water bath within shaker at temperature 75 °C during 30 minute, continue by sentrifugation until two layer solution yielded. The upper layer was taken and ready to be injected to GC-MS instrument. One microliter fatty acid methylene ester that have been prepared is also injected to GC-MS instrument. The operation conditions of chromatography device were suitable to fatty acid methylene ester. The resulted chromatography peaks were scanned in order to read the mass spectra that then interpreted to determine the type of molecule of every peak.

#### Examining repeatability and reproducibility

Repeatability is the ability of the method to produce the same data for the same replication of sample, operator, device, place and condition. Repeatability was examined by applying the method to analyse a sample at a laboratory by the same operator in the same day. Thus, reproducibility is the ability of the method to produce the same data of the same sample with different operator, device, place and condition. Reproducibility was evaluated by applying developed method to analyse a sample in some laboratories with different operator

**Table 1.** Omega-3 content in oilfish based on alkalimetric titration

Analysis place	Average omega-3 content (%w/w)	Standard Deviation	Bias (%)	Relative errors (%)
Organic Lab. UNY	24.47	0.0286	4.78	0.12
Organic Lab. UGM	20.54	0.6866	20.08	3.34
General Chem. Lab. UNY	24.23	1.4208	5.72	5.81
Borobudur Lab.	25.25	0.3150	1.75	1.25
Average	23.62	<b>0.6128</b>	<b>8.08</b>	<b>2.63</b>
Standard Deviation	1.8192			
Repeatability		97.37%		
Reproducibility		92.3%		

and time. Repeatability and reproducibility of the developed method were then compared to label content of fish oil capsules. Total amount of omega-3 content in fish oil capsules label is 25.7%. %bias was evaluated with comparing total omega-3 in fish oil capsules label content with standard method result by GC-MS.

## RESULT AND DISCUSSION

Results of the research are shown in Table 1.

#### Analysis result by Gas Chromatography – Mass Spectroscopy

Analysis by Gas Chromatography – Mass Spectroscopy of the same sample has already done. Chromatogram of analysis result has shown in enclosure, where the data will be used as comparator. The result showed omega-3 content such as:

Linoleic acid : 3.41%  
 DHA : 10.18%  
 EPA : 15.48%  
 Total : 29.07%

If compared to label content of fish oil, it can be evaluate that error (bias) as:

$$\begin{aligned} \% \text{ Bias} &= \frac{\text{analysis result} - \text{label content}}{\text{label content}} \times 100\% \\ &= \frac{29.07 - 25.7}{25.7} \times 100 \% \\ &= 13.11\% \end{aligned}$$

The analysis was use liquid paraffin as solvent, which is an alkana compound so it's very stabil from oxidation and high boiling point effects. The purpose of paraffin inclusion is to complet the reaction without producing the bothering side product and will not be distilled.

The problem that has been faced was there a bumping in distillation process, because the existences of hot organic layer at the liquid surface that hited by water drop. The bumping was then overcome by filtering the reflux resulted liquid to get clear liquid

that is free from MnO<sub>2</sub> sediment and before distilled paraffin.

Distillation process is a most potential phase to give error value of the analysis, because distillation is a process that relative controlled. Every device will provide different separation process, depend on device shape and size, cooler (condenser) length and flow rate of water cooler. The analyst has also had different perception for distillation ending point determination. An inaccurate ending point will give an inappropriate data.

Using the recovery factor can minimize lots of thing that influence of distillation accuracy. Recovery factor was measured by distilling propanoic acid with known amount at the same distillation condition. Distillation result was then titrated and compared to the same amount of undistilled propanoic acid. The difference value is the recovery factor to correct that analysis result. Recovery factor might be determined for applying different device and/or operator.

Based on result data and calculating data, it was known that the previous developed method in this research could be use as analysis method of omega-3 content in an oil sample. The method results low error. Average relative error was 2.63%, whereas average method error was 8.08%. Relatif error of repeatability for every laboratory are in range of 0.12 - 5.81 with average 2.63%. Thus, the reproducibility that gated from deviation standard and average analysis result from three laboratories was 92.3%.

Analysis result of the developed method compared to GC-MS analysis result has also give a good result. GC-MS method has an advantage to know omega-3 composition contained, but it was difficult in quantitative calculation. Resulting chromatogram was easier to calculate relative percentage of fatty acid component, whereas content calculation of the chromatogram was difficult because it should use standar and calculation factors. Calculation result was showed the omega-3 content of 29.07% with error percentage of 13.11%.

Error percent of the developed method (8.08%) is better than the GC – MS method (13.11%). The higher of GC – MS error was caused by lots of probabilities of error, such as process of preparation, dilution, injection and also arrangement of device condition.

Further improvement of developed method is still need to be done until the method can be use as standard method. The improvement is prior in distillation device standardization, including device shape and size. Beside that, distillation process is also need to be standardized in order to get the same end of distillation process so the analysis result will be more valid.

Standardisation process can be done by standardization of heater, distillation rate and distillate volume. Device and distillation process standardizations can be relate to distillation method of Rheicert-Meisel in determination of volatile fatty acid in oil sample.

## CONCLUSION

Omega-3 fatty acid content can be analysed by alkalimetric titration through propanoic acid derivate formation. The method was modified in this research in order to completion including organic solvent substitution, liquid KMnO<sub>4</sub> usage and recovery factor inclusion.

The new developed method in this research showed good accuracy and precise to analyse known content. Accuracy was expressed as bias percentage in average of 8.08%, whereas precise was expressed as relative error in average of 2.63%. Accuracy of this developed method was better than GC-MS method that resulting bias percentage of 13.11 %. Analysis in every laboratory showed good repeatability with relative error in range 0.12 – 5.81%. Applying the method at different laboratory showed good reproducibility with relative error of 7.70%.

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