Determination of Docosahexaenoic Acid (DHA) In Food Supplement Using Gas Chromatography

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Determination of Docosahexaenoic acid (DHA, C22:6 ω-3) in two registered food supplement using Gas Chromatography (GC) have been performed. These food supplements were soft capsules containing natural fish oil that have been claimed ‘providing with DHA’. The aim of this study was to determine DHA content in natural fish oil soft capsules. Sample was hydrolyzed with methanolic NaOH and converted to DHA-methyl ester using methanolic BF₃ as catalyst. The obtained DHA-methyl ester was extracted with n-heptane and analyzed by GC using tricosanoic methyl ester as internal standard. The present work showed that all validation parameters obtained were fulfill to Association of Official Analytical Chemist (AOAC) criteria for methyl esters of fatty acids in oils and fats analysis, i.e. Rs>1.25, good linearity (r²=0.9988, Vxo=3.85%), good precision (CV<10%) and good accuracy (recoveries was 95.79%). The optimum conditions of GC obtained as follow, inlet and detector temperature was 250°C and 300°C respectively. Oven temperature was programmed at 180°C (1 minute), to be ramp at 10°C/minute to 280°C (3 minute). Helium’s flow rate was 1.1 mL/minute. The DHA contents in each capsule of sample X and sample Y were found to be=219.0mg (CV=0.88%) and 139.2mg (CV=1.91%), respectively. These DHA’s contents in each capsule were higher than that mention in their leaflet i.e. 95 mg and 120 mg, respectively.

Keywords: Docosahexaenoic acid, DHA, food supplement, GC

INTRODUCTION

Docosahexaenoic acid (DHA) (Fig.1) is essential substance for human being. Therefore, some different kind of food products was enriched with DHA. Particularly in encapsulated food supplement, DHA was added as the essential contents of natural fish oil, and claimed as nutritional supplement for pregnant women or infants. The DHA content in encapsulated sample must be controlled because of many reasons such as variation of DHA content in natural fish oil and DHA deterioration process. DHA is deteriorating rapidly when exposed to light, air and heat. Furthermore, control on DHA content in foods product can prevent over consumed of DHA (The Analyst™, 2002).

The aim of this study were determining the concentration of DHA in two registered food supplement products which relatively wide distribute in Indonesia using Gas Chromatography (GC).

![Figure 1. Docosahexaenoic acid](image)

MATERIALS AND METHOD

All chemicals were of pro analysis grade, unless otherwise noted, i.e. DHA (Docosahexaenoic acid), TME (Tricosanoic Methyl Ester), heptane, NaCl, NaOH, Na₂SO₄ anhydrous, methanol, BF₃ 12% in methanol.

Samples to be analyzed were two food supplements which have registered number of POM BTI (code=X) and ML (code=Y). These two samples were obtained from pharmacies in Surabaya.

Instrument that have been used were GC (Agilent 6890 series) equipped with capillary column (30.0 m, id 320μm, fc 0.25μm, using HP-5 5% Phenylmethylsiloxane as stationery phase), Helium as carrier gas, FID for DHA peak quantization and GC (HP 5890 series II) equipped with HP1 column (25m, id 0.2mm, fc 0.25μm) with MSD 5972 as instrument for confirmation of DHA peak.

Reagents preparation and esterification procedures were adopted from AOAC Official Method for Fatty Acids in Oils and Fats (Cunnif, 1995). Esterification procedure was as follows: aliquots DHA standards solution (or sample solutions) that have been dried previously in the glass tube was added with 400μL of 2000 ppm TME standard solution (as Internal standard) and the mixture obtained to be dried in gentle stream of N₂. Furthermore, 1.5mL of 0.5 N methanolic NaOH was added, then blanket with N₂, cap, mixed and heated for 5 minute at 100°C in the water bath. After the mixture was cooled, 2mL BF₃ in methanol was added and blanket again with N₂, cap tightly, mixed and to be heated for 30 minute at 100°C in the water bath. The mixture obtained was cooled to (30-40)°C, 2mL heptane was added, blanket with N₂, cap, and shake vigorously for 30 second while still warm. Immediately 5mL of saturated NaCl solution was added, blanket with N₂, cap and agitated thoroughly. Finally, the mixture obtained cooled to room temperature. When heptanes layer separates from aqueous lower phase, heptane layer was transferred to a clean glass tube. (Blanket this heptane layer with N₂ and cap if not to be injected immediately). 1μL heptanes layer was injected into GC system.

The method was validated for optimation of GC condition in separating DHA from another substances in sample simultaneously, i.e. injector, oven and detector temperatures, split ratio, carrier gas flow rate
etc. Identification of DHA in sample based on the similarity of shape and retention time ($t_R$) of suspected DHA’s peak in the sample with standard DHA. Spiking with DHA standard and determination DHA peak with GC-MSD were used for confirmation of DHA peak.

**RESULT AND DISCUSSION**

The optimum conditions of GC obtained were as follow: inlet and detector temperature was $250^\circ C$ and $300^\circ C$ respectively. Oven temperature was programmed $180^\circ C$ (1 minute), ramp $10^\circ C$/minute to $280^\circ C$ (3 minute). Helium’s flow rate was 1.1 mL/minute and split ratio was (1:10). Chromatograms of methyl esters of standard DHA, sample X, sample Y were showed on figure 2, 3 and 4 respectively.

![Figure 2. Chromatogram of methyl esters standard DHA, EPA and LNA](image)

![Figure 3. Chromatogram of fatty acid methyl esters of sample X](image)

![Figure 4. Chromatogram of fatty acid methyl esters of sample Y](image)

Identification of DHA in sample X ($t_R=28.40$ minute) or in sample Y ($t_R=28.39$) based on the similarity of retention time with standard DHA ($t_R=28.39$ minute). There was increasing area at suspected DHA peak when spiked sample had been injected. The GC-MSD data peak of suspected DHA methyl esters in sample match to standard DHA with 99% match factor grade. Figure 3 and figure 4, showed good resolutions ($Rs>1.50$) were obtained between DHA and another substances peaks. This results fulfilled with AOAC criteria for Methyl Esters of Fatty Acids in Oils and Fats Analysis, i.e. $Rs>1.25$ (Cunnif, 1995).

According to linearity test, peak area of DHA methyl ester showed a linear correlation with relative response of detector ($r_{\text{calculated}}=0.9988$ more than $r_{\text{table}}=0.878$ with $\alpha=0.05$, $p=4$). So, DHA concentrations can be determined in the range of (250-2000) ppm with good result ($Y=1.0606.10^{1X}-0.1858$, $S_{\text{r}}=4.271.10^{-2}$, $S_{\text{m}}=40.27$, $V_{\text{x}}=3.83\%$). Internal standard (TME) must be added simultaneously at each determination as a correction factor in extraction and injection sample process.

Precision factor of DHA analysis was showed by CV data of DHA standard methyl ester’s signal (1000 ppm) when of injected 10 times, i.e. 5.93% (without internal standard) or 1.81% (when injected simultaneously with 400 ppm TME as internal standard). This result was fulfilled with AOAC criteria i.e.<10 % (Cunnif P., 1995).

Precision of DHA esterification and extraction processes were determined using a mixture of standard DHA (500 ppm) and TME (400 ppm) (five time replication). The data showed that TME could improved CV obtained i.e. 7.65% in compared with CV 24.89% (if standard DHA was processed without TME as internal standard). This data showed that internal standard must be added in each replication. Fluctuation of esterification process in glass tube and completeness of extraction process would influenced those deviation.

Accuracy method test was performed using fortified procedure (Cunnif, 1995). Recoveries obtained were 95.79%, with CV of 6.43%. This percentage recovery was fulfilled with standard AOAC criteria (i.e.80-120%).

Results of determination of DHA in sample X and sample Y were showed in table 1 as follow.

The DHA concentration in sample X was higher (230%) than that mention in its leaflet (i.e. 95 mg per capsule). This high concentration was supposed to be caused by variation in DHA concentration of natural fish oil used in each batch of production. As a matter of fact, oil that was extracted from deep sea fish (e.g. Cod oil) containing higher concentration of DHA. Moreover, there was other kind of oil which mention in this product leaflet supposed to contain DHA too (but not mention in the leaflet).

**Conclusion**

Method that was used in determining DHA concentration in food supplement sample was fulfilled with the standard validation criteria. The concentration of DHA in sample X and sample Y were 219.0 mg/capsule and 139.2 mg/capsule respectively. These obtained concentrations were higher than DHA concentrations mention in their leaflet.
Table 1. Result of the determination of DHA in sample X and sample Y

<table>
<thead>
<tr>
<th>Replication</th>
<th>DHA cons. (%)</th>
<th>DHA Cons (mg)/capsule</th>
<th>DHA cons. (%)</th>
<th>DHA Cons (mg)/capsule</th>
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<tr>
<td>1</td>
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<td>13.50</td>
<td>138.9</td>
</tr>
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<td>218.1</td>
<td>13.45</td>
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</tr>
<tr>
<td>6</td>
<td>42.60</td>
<td>220.3</td>
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</tr>
<tr>
<td>Mean</td>
<td>42.43</td>
<td>219.0</td>
<td>13.54</td>
<td>139.2</td>
</tr>
</tbody>
</table>

CV = 0.88% CV = 1.91

Note: Mean weight per capsule (X) was 0.5168 gram and capsule (Y) was 1.0277 gram

REFERENCES


