VALIDATION OF HPLC METHOD FOR DETERMINATION OF DOCOSAHEXAENOIC ACID (DHA) IN MULTIVITAMIN CAPSULE

DIAH WIDOWATI*, ROS SUMARNY, ESTI MUMPUNI

Fakultas Farmasi Universitas Pancasila
Jln. Srengseng Sawah, Jagakarsa, Jakarta Selatan 12640

ABSTRACT

Current studies show that there are no harmful affects of multivitamin supplement preparation with DHA (docosahexaenoic acid). Multivitamin preparation containing various substances of varying characteristics may have problems in quantitative analysis. High performance liquid chromatography (HPLC) due to its high capability in separating a mixture of substances is applicable for determining each component of multivitamin preparation. This research has developed a validated HPLC method for determining DHA in soft-capsule of multivitamin-mineral. The chromatographic separation was achieved with a C8 column as stationary phase and a mixture of acetonitrile-water-methanol (70:20:10) as mobile phase with the flow rate of 0.7 mL/minute. The effluent was monitored at 23 nm. Effective separation and quantification was achieved in less than eight minutes. The method was simple, precise, accurate and has been successfully applied for the determination of DHA in soft-capsule of multivitamin-mineral.

Keywords: DHA, HPLC, validation

INTRODUCTION

Docosahexaenoic acid (DHA) are widely known as it is commonly added to many products of infant formula in order to enhance brain development and eyesight of babies. Currently there is not only used for infant formula, but DHA also added to the multivitamin preparations, especially for children and pregnant/lactating women. Multivitamin preparation that contain DHA are generally formulated as oral solution, but recently it was also developed in capsule form, either soft shell capsules or hard shell capsules. To ensure the quality of the multivitamin preparations, validated quality testing method. One of the quality testing method is the determination of the DHA as active compounds. Determination of DHA in a multivitamin preparation is generally carried out by gas chromatography. As an alternative method, a high performance liquid chromatography (HPLC) method has been developed, i.e a reverse phase
HPLC to determine DHA in soft capsules of multivitamin-mineral preparation. Validation parameters included selectivity, precision and accuracy.

EXPERIMENTAL METHOD

1. Apparatus
HPLC Shimadzu LC-20AD with UV-detector at 237 nm using Hypersil Gold Thermo C8 (250 X 4.6 mm ;5µm), Analytical Balance (Mettler ab204s)

2. Chemicals and Reagents
DHA-S (vegetarian), DHA content 350m9/g acetonitrile (Mallincrodt), methanol (Mallincrodt), chloroform (Merck)

3. Method
3.1. Preparation of Standard Solution
Transfer about 75 mg of DHA, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with a mixture of chloroform methanol (2:1) to volume, and mix. Transfer 5.0 mL of this solution to a second 10-mL volumetric flask, dilute with acetonitrile to volume and mix.

3.2. Assay Preparation
Transfer an accurately weighed portion of capsule contents, equivalent to about 75 mg of DHA to a 10-mL volumetric flask, dissolve in and dilute with a mixture of chloroform-methanol (2:1) to volume, mix and filtered. Transfer 5.0 mL of the clear filtrate to a second 10-mL volumetric flask, dilute with acetonitrile to volume, and mix.

4. Results
The best result of reversed phase HPLC method for determination of DHA was obtained with the used of a 5µm C8 column of (250 x 4.6 mm) dimension. A mixture of acetonitrile-water-methanol (70:20:10) was used as mobile phase with flow rate of 0.7 mL/minute. The effluent was monitored at 237 nm. Effective separation and quantification was achieved, the retention time of DHA is at 5.5 minute and the resolution value is 1.8 . The retention time less than 10 minutes indicated an efficient method and the resolution value more than 1.5 indicated good separation.
Metode Validation.
According to the International Conference on Harmonization (ICH), method validation was performed to ensure that an analytical methodology were systemically suitable, accurate, specific, and reproducible over the specified concentration range of the analyte. The performance qualification of HPLC was determined with the system suitability test to verify system performance under actual running condition with a well-characterized analyte mixture, column and mobile phase. The system suitability for five times injection conformed with the specification of coefficient of variation (CV) of < 2%. The linearity of the method was determined by standard addition method to get concentration between 50
% and 150% of the expected concentration. The linearity of DHA was obtained with correlation coefficient above 0.99 and the regression equation is $y = -51757 + 375.99x$

Figure 3. Linearity of DHA

<table>
<thead>
<tr>
<th>Sample 70%</th>
<th>DHA Standard</th>
<th>Sample + DHA Standard</th>
<th>Area</th>
<th>mg</th>
<th>%</th>
<th>Average (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45,4913</td>
<td>14,3864</td>
<td>80% 59,8777</td>
<td>1079218</td>
<td>14,4500</td>
<td>100,4424</td>
<td>101,3853</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100% 74,2841</td>
<td>1328625</td>
<td>28,3025</td>
<td>98.3654</td>
<td>99.3048</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120% 88,6505</td>
<td>1593556</td>
<td>43,0171</td>
<td>99.6708</td>
<td>99.2401</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Average 99.9767 1.08

Figure 4. Recovery of DHA
The accuracy and precision of the method was indicated by the values of recovery and CV. The accuracy was carried out by standard addition method. The average values obtained for recovery (99.98 \%) and the CV (1.08\%), showed the accuracy and reproducibility of the method meet the specification.

5. Conclusion
The developed HPLC method using C8 column of (250 X 4.6 mm) dimension and 5um of particle size, a mixture of acetonitrile-water-methanol (70:20:10) as mobile phase with flow rate 0.7 mL/minute, using UV detector at 237 nm was simple, accurate, precise and could be successfully applied for the analysis of DHA in soft capsules of multivitamin-mineral preparation.

Acknowledgement
We are grateful to Ministry of National Education Republic of Indonesia for financial support and to Faculty of Pharmacy University of Pancasila for providing laboratory facilities.

References
4. International Conference on Harmonization, validation of analytical procedures: text and methodology Q2 (R1). 2005