Column Chromatographic Isolation of Docosahexaenoic Acid from Fish Oil and its Assessment by Analytical Techniques

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ABSTRACT

Docosahexaenoic acid (DHA) was isolated from fish oil and identified by gas chromatography. The isolation of DHA was carried out by the column chromatography. The preparation of column using silica gel 60-120 mesh and elution of column with different solvent in increasing order of polarity was performed. Isolated fractions were subjected to identification test for DHA by thin layer chromatography using solvent benzene. Isolated DHA was then subjected to high performance thin layer chromatography using chloroform as solvent. Determination of isolated DHA was carried out by gas chromatography using hexane as a solvent. The chromatogram of isolated DHA and standard DHA were compared. It was showed that in thin layer chromatography the fraction 51-58 and 72-85 showed single spot in benzene mobile phase having Rf value 0.56 when compared with standard DHA which matched with Rf Value of standard DHA. The retention time of isolated DHA and standard DHA by gas chromatography were 5.73 and 5.01 respectively. Isolated DHA showed 95.21 percentage purity. It was observed that fish oil can be used as precursor of DHA.

INTRODUCTION

The health benefits of fish oil include its ability to aid in treatment of heart diseases, high cholesterol, depression, anxiety, low immunity, cancer, diabetes, inflammation, arthritis, AIDS, Alzheimer's disease, eye disorders, macular degeneration and ulcers. It also helps in weight loss, pregnancy, fertility and skin care (particular for disorders such as psoriasis, acne).[1] Most of these health benefits of fish oil can be attributed to the presence of Omega 3 essential fatty acids such as Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). The various types of fish which can be a good source of fish oil are mackerel, rainbow trout, lake trout, halibut, herring, sea bass, sardines, swordfish, oysters, albacore tuna, bluefin tuna, yellowfin tuna, turbot, pilchards, anchovies and salmon.[1,9]

Chromatography

Chromatography is the process for the separation of molecular mixtures by distribution of the solutes between two phases, the phases being contacted in a continuous countercurrent manner.[2]

Column chromatography

Column chromatography is the method used to purify individual compounds from mixtures of compounds between two phases. In column chromatography, the stationary phase is a solid substances such as silica gel, alumina etc. It should not react with the substance to be separated and insoluble with the solution under test and solvent. For good separation, the component of the mixture should have different degree of affinity for the solid support. The mobile phase is a different polar and non-polar solvent. There is an optimum flow rate for each particular separation. The sequence of solvents used in the increasing polarity.[3]

Gas chromatography

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance or separating the different components of a mixture (the relative amounts of such components can also be determined). In gas chromatography, the mobile phase (or "moving phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase

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is a microscopic layer of liquid or polymer on an inert solid support inside a piece of glass or metal tubing called a column. The instrument used to perform gas chromatography is called a gas chromatograph.[3] The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.

MATERIALS AND METHODS:

DHA was supplied by Central India Pharmaceuticals, MIDC, Nagpur. Fish oil was obtained from Janta Aqua Fisheries Udupi, Karnataka. Other chemicals were of analytical grade. UV spectrophotometer (UV 1700 Shimadzu Japan), Gas Chromatograph (Thermo scientific Trace GC 600 with fused silica capillary column), HPTLC with CAMAG high-tech UV cabinet fitted with dual wavelength 254/366 nm, 8 volts UV lamp were used in this study.

Isolation of DHA from fish oil by column chromatography:

As fish oil has higher concentration of DHA, it was used for isolation and the procedure was carried out by column chromatography. For every solvent or solvent mixture, 30 ml of each fraction was collected in a conical flask, distilled to remove solvent, concentrated fraction was collected in a test tube. Then concentrated fractions were subjected to thin layer chromatography by using different solvent system and 50% H_2SO_4 and iodine as detecting reagents.[4]

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Table1. Column chromatography for separation of DHA from fish oil

Sr. No.	Solvents used for elution	Fraction no
1	Petroleum ether	1-6
2	Petroleum ether : n-hexane (90:10)	7-14
3	Petroleum ether : n-hexane (80:20)	15-21
4	Petroleum ether : n-hexane (70:30)	22-29
5	Petroleum ether : n-hexane (60:40)	30-37
6	Petroleum ether : n-hexane (50:50)	38-44
7	Petroleum ether : n-hexane (40:60)	45-50
8	Petroleum ether : n-hexane (30:70)	51-58
9	Petroleum ether : n-hexane (20:80)	59-66
10	Petroleum ether : n-hexane (10:90)	67-71
11	n-hexane	72-76
12	n-hexane : Chloroform (99:1)	77-85
13	n-hexane : Chloroform (98:2)	86-91
14	n-hexane : Chloroform (97:3)	92-101
15	n-hexane : Chloroform (96:4)	102-111
16	n-hexane : Chloroform (95:5)	112-121
17	n-hexane : Chloroform (94:6)	122-130

Thin layer Chromatography of Isolated compound:

Chromatographic pattern of the isolated compound was studied by thin layer chromatography.

Table 2: Thin layer Chromatography of Isolated DHA

Sr. No.	Sample spots applied	Solvent system used	No. of spots with different locating agent			R_f value
			UV	Iodine	50 % H_2SO_4	
1.	Isolated DHA	Benzene	---	1	1	0.56

R_f value of standard DHA: 0.56

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Analysis of isolated DHA:

Analysis of isolated DHA was done using UV spectrophotometer by determination of λ_{max}.[5]
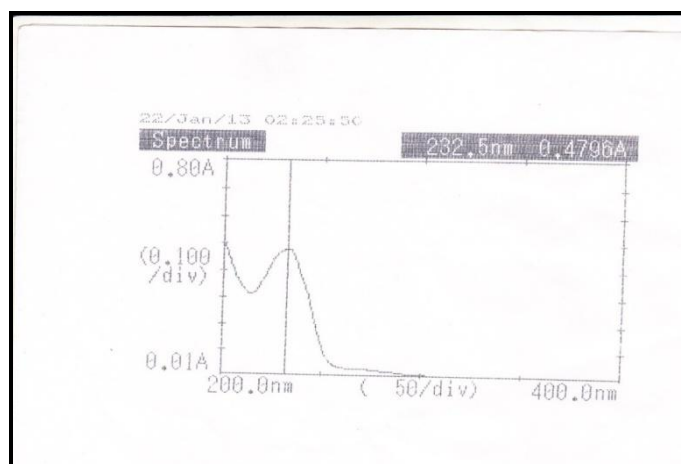

Figure 1: UV spectra of isolated DHA

Different dilutions of isolated DHA i.e., 10 to 50 μg/ml were prepared using n-hexane. The absorbance of each of the solution was measured at 232.5 nm in a 1 cm cell against the solvent blank and a graph was plotted. The graph showed that drug follows Beer-Lambert law.

Table 3: Standard calibration data of DHA

| Sr. No.	Concentration (μg/ml)	Absorbance
1	10	0.1532
2	20	0.3577
3	30	0.4570
4	40	0.5961
5	50	0.7631

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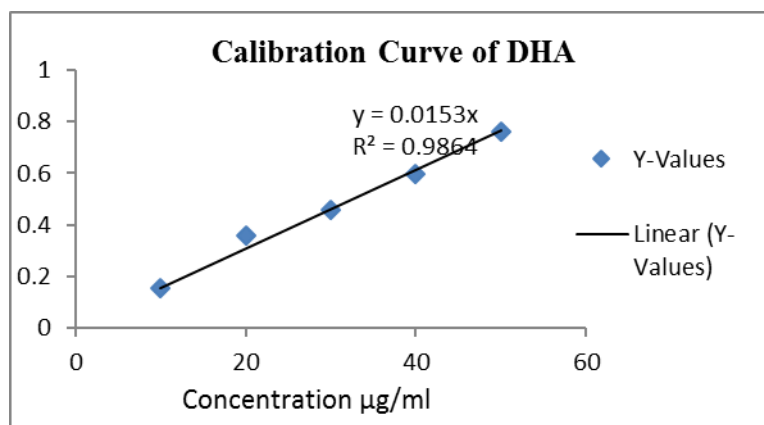
Fig 2: Calibration Curve of DHA

## HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY [6]

Standard solution: An accurately weighed 10 mg of DHA was transferred to 25 ml volumetric flask and was dissolved in chloroform. The volume was made up by chloroform to have final concentration (0.4µg/ml).
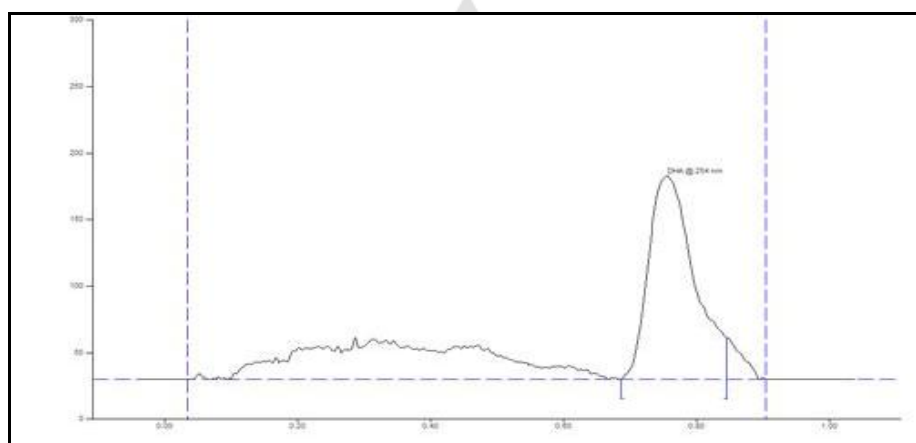
Fig 3: Chromatogram of standard DHA

Results of HPTLC of standard DHA

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.69	0.5	0.76	153.1	100.00	0.85	31.5	9079.8	100.00

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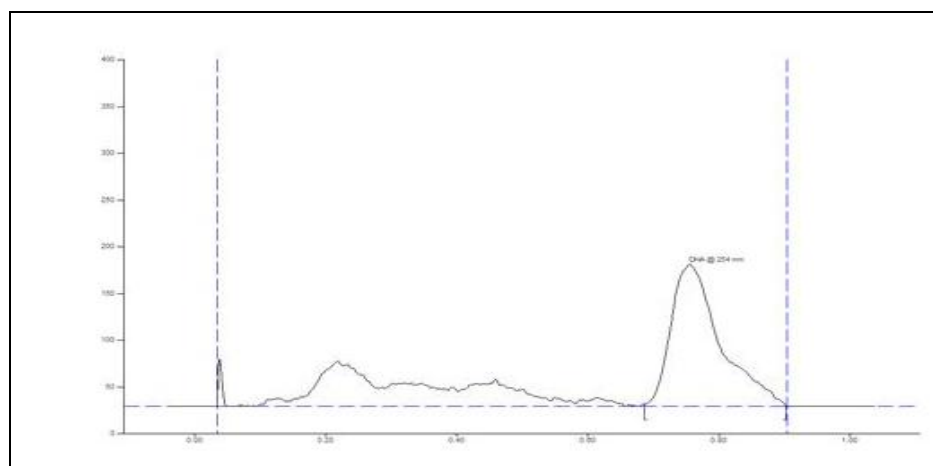
Fig 4: Chromatogram of isolated DHA

Results of HPTLC of isolated DHA

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.69	2.6	0.76	152.1	100.00	0.90	0.9	9861.9	100.00

DETERMINATION OF ISOLATED DHA BY GAS CHROMATOGRAPHY[7,8]

Instrument	Thermo scientific Gas Chromatograph
Model no	Trace GC 600
Column	fused silica capillary column
Column dimension	30 m X 0.25 mm ID X 0.25 μm film thickness
Carrier gas	Nitrogen
Detector	Flame ionization detector
Flow	1-2ml /minutes
Injector mode	Split
Pressure	73 kpa
Oven temperature	110°C
Injector volume	1μl
Injector Temperature	70°C
Oven Temperature	190°C
Run Time	30 min

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Preparation of Standard solution

An accurately weighed 25 mg of standard DHA was dissolved in sufficient quantity of chloroform in 25 ml of volumetric flask. Then, 1.0µl of sample was injected manually into injector of gas chromatography.
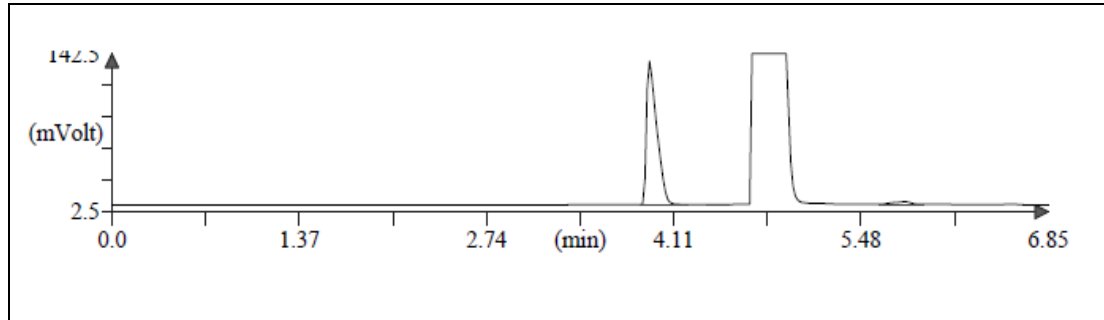
Fig 5: Chromatogram of standard DHA

Sr. No.	Peaks	Drug sample	Ret time (min)	Area (µ*V*Sec)	Peak height
1	1	Chloroform	3.933	6629819	127235
2	2	Standard DHA	5.011	200202	2332

Preparation of test solution:

An accurately weighed 25 mg of isolated DHA was dissolved in sufficient quantity of chloroform in 25 ml of volumetric flask. Then, 1.0 µl of sample was injected manually into injector of gas chromatography.
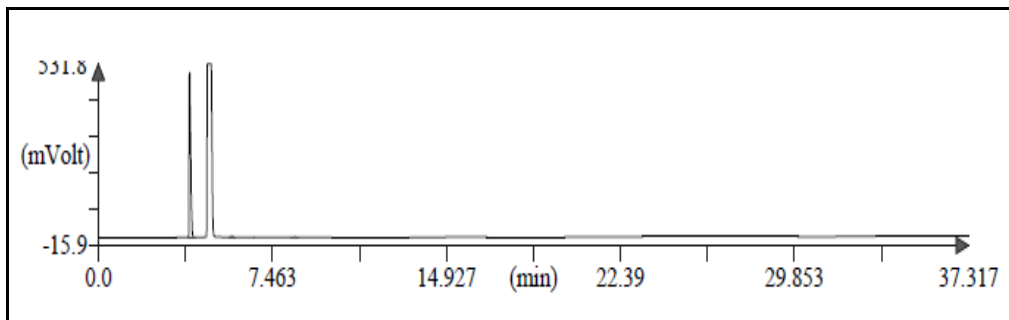

Fig 6: Chromatogram of isolated DHA

Sr. No.	Peaks	Drug sample	Ret time (min)	Area (µ*V*Sec)	Peak height
1	1	Chloroform	3.917	2312315	497961
2	2	DHA oil	5.733	190621	2559

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Calculation:

Calculation % Assay by using formula.

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times 100$$

Where,　　　AT= Peak area of sample injection

　　　　　AS= Peak area of standard injection

　　　　WS= Weight of working standard taken in mg

　　　　WT= Weight of sample taken in mg

　　　　DS= Dilution of standard

DT= Dilution of sample

$$\% \text{ Assay} = \frac{190621}{200202} \times \frac{0.02053}{20} \times \frac{20}{0.02047} \times 100$$

= 95.21%

Percentage purity of isolated was found to be **95.21%**

DISCUSSION AND CONCLUSION

DHA was isolated from fish oil by column chromatography by using different solvents in increasing order of polarity. Mobile phase constitutes of Benzene showed maximum resolution and reproducible results. The Docosahexaenoic acid showed one spot and R_f value of Docosahexaenoic acid was found to be 0.56. The isolated oil showed the UV spectrum at 232.5 nm (fig. 1) and from the standard calibration curve of isolated DHA (table 3) in n-hexane, it was concluded that drug obeys Beer-Lambert's law. From the gas chromatography analysis, the percentage purity of isolated compound was found out to be 95.21%.

REFERENCES

1. http://en.wikipedia.org/wiki/fish oil (Cited on 2012 Aug 1)
2. Skoog DA, Holler FJ and Nieman TA. Principles of instrumental Analysis. 5th ed. a division of Thomason Learning, Inc.; 2004; p. 674-677.
3. Chatwal GR & Anand SK. Instrumental methods of chemical analysis. 5th ed. Himalaya publishing house; 2010; P.264-267.
4. Stahl E. Thin layer chromatography. 2nd ed. Springer-Verlay Berlin Publisher; 2005; 2-10.

Citation: Rasika R. Nikhade et al. Ijppr.Human, 2017; Vol. 9 (1): 49-58.

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5. Silverstein RM, Spectrometric identification of organic compound. 7th ed. California A Wiley-Interscience Publication, 2005; 2-200.

6. Sethi PD. HPTLC Quantitative Analysis of Pharmaceutical Formulations. CBS Publishers and Distributors, New Delhi: 1996; p.5-7.

7. Tande T, Breivek H, Validation of a method for gas chromatographic analysis of eicosapentaenoic acid and docosahexaenoic acid as an active ingredient in medicinal products. JAOCS 1992; 69:1124-30.

8. Fournier V, Destaillats F, Hug B. Quantification of eicosapentanoic acid and docosahexaenoic acid geometrical isomers formed during fish oil deodorization by gas-liquid chromatography. J Chromatography a 2007; 1154:353-59.

9. Horrocks LA, Yeo YK. Health benefits of docosahexaenoic acid (DHA). Pharmacol Res1999; 40:211-25.

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