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ISOLATION OF ALPHA LINOLENIC ACID FROM LINSEED OIL AND ITS IDENTIFICATION BY GAS CHROMATOGRAPHY

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ABSTRACT

The alpha linolenic acid (ALA) was isolated from linseed oil and identified by gas chromatography. The isolation of ALA 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 done. isolated fractions were subjected to identification test for ALA by thin layer chromatography using solvent benzene and methanol (95:5). Determination of isolated ALA was carried out by gas chromatography using reagent hexane as a solvent. The preparation of standard and test solution of LA was prepared in chloroform. The chromatogram of isolated ALA and standard ALA were compared. It was showed that in thin layer chromatography the fraction 10-16 showed single spot in benzene and methanol(95:5 ratio) mobile phase having R_f value 0.86 when compared with standard ALA which matched with R_f Value of standard ALA. The retention time of isolated ALA and standard ALA by gas chromatography were 18.83 and 17.981 respectively. Isolated ALA showed 80.74 percentage purity. It was observed that besides fish oil, plant oil can be used as precursor of ALA. It was observed that the developed method of column chromatographic isolation of ALA from linseed oil is simple, accurate and precise.

INTRODUCTION

Alpha linolenic acid(ALA) is polyunsaturated fatty acid. It can not be synthesized by human body therefore must be obtained from food and other dietary sources and are hence called essential fatty acid. ALA is widely distributed in plant oils. Linseed oil is one of the source to isolated ALA. Fish oil is also source of long chain polyunsaturated fatty acids fish oil contain omega-3 fatty acids, eicosapentaenoic acid, docosahexaenoic acid but it is non-acceptable for vegetarian people. The present study deals with the isolation of ALA by column chromatography and its identification by gas chromatography. This method is used for separation of gases and volatile substances which are difficult to separate and analyse. It is also useful for estimation of organic volatile impurities, analysis of various pharmaceutical products and drugs**.** The non –fish eaters, there is no source of long chain polyunsaturated fatty (LCPUFAs) acid except than fish oil. An alternative source of LCPUFAs production, plant oils can be used as precursor of ALA So aim of the study, to isolate ALA from linseed oil and analysis by gas chromatography.

MATERIALS AND METHODS

ALA was supplied by Central India Pharmaceuticals, MIDC, Nagpur. Other chemicals were of analytical grade. UV spectrophotometer (UV 1700 Shimadzu Japan), Gas Chromatograph (Thermo scientific Trace GC 600 with fused silica capillary column),

Isolation of ALA from linseed oil by Column Chromatography

Isolation of ALA from Linseed oil was performed as it has higher concentration of ALA. The isolation procedure was carried out by column chromatography as follows.

Preparation of column

The borosilicate glass column was used. The column was cleaned thoroughly using chromic acid and washed with distilled water till free from acid. Then it was rinsed with acetone and dried. Silica gel 60-120 mesh was activated at 120˚C in hot air oven for 1 hr was mixed with petroleum ether to get slurry. The column was fixed vertically on a stand; a cotton plug was inserted to the bottom of the column, filled with petroleum ether.

The slurry of silica gel was poured slowly from the top. After all silica had settled a plug of cotton was placed over it. The solvent used for elution was maintained 10 cm above the cotton plug. Linseed oil was triturated with small quantity of silica gel and poured from top of the column; a plug of cotton was placed over it and solvent was maintained 10 cm above the cotton plug.

Elution of column

The column was eluted successively with different solvents and mixture of solvents in increasing order of polarity. Eluted fractions were subjected to thin layer chromatography.

Thin layer Chromatography of Isolated compound

TABLE 1: THIN LAYER CHROMATOGRAPHY OF ISOLATED ALA

Sr.No	Sample spot applied	Solvent system used	Number of spots with		R_f value
			Iodine	H_2SO_4	
1	Isolated ALA	Benzene and methanol	1	1	0.86

Determination of isolated ALA by gas chromatograph

Chromatographic condition:

TABLE 2: CHROMATOGRAPHIC CONDITIONS

Column	Omega wax 250 fused silica capillary column
Column dimension	30 m X 0.25 mm ID X 0.25 µm film thickness
Carrier gas	Nitrogen
Flow	1-2ml /minutes
Injector mode	Split
Injector temperature	200˚C
Injector volume	1.0 µl
Detector Temperature	205˚C
Oven programming	
Temperature	240˚C
Hold time	20 min
Total run time	25 min

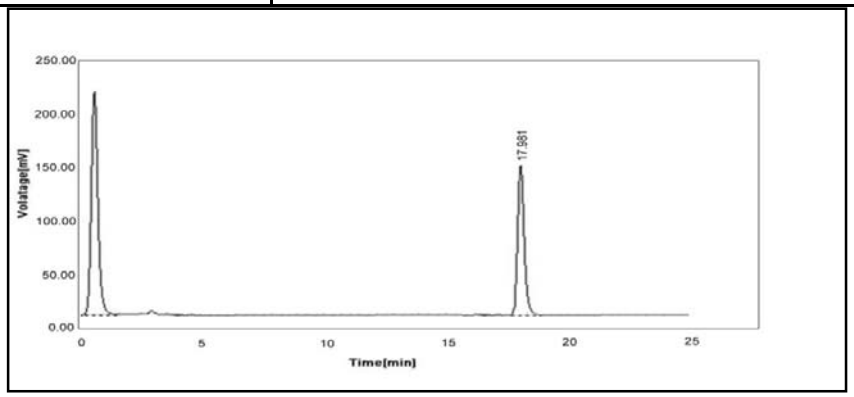

FIG1: CHROMATOGRAM OF STANDARD ALA SOLUTION

TABLE 3:CHROMATOGRAPHIC OBSERVATIONS OF STANDARD ALA SOLUTION.

Peak no.	RT[min]	Area[mV*s]	Height[mV]	Area[%]	Height[%]	Wo5[min]
1	17.981	75.654	1.957	100.0	100.0	0.71

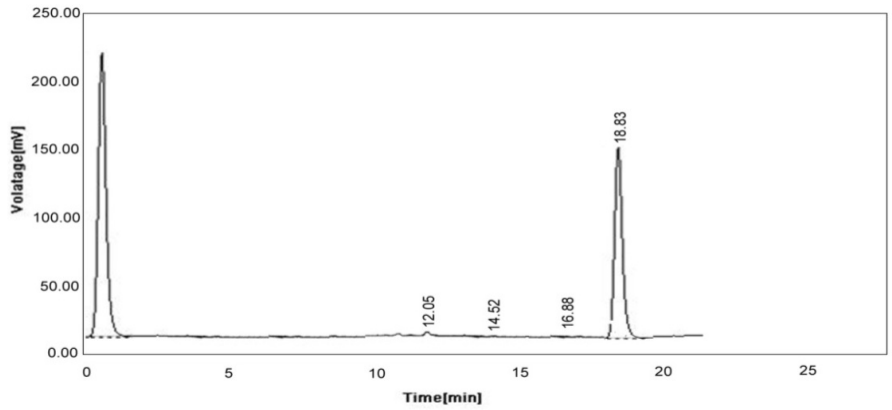

FIG2: CHROMATOGRAM OF ISOLATED ALA SOLUTION.

Peak no.	RT(min)	Area(mV*s)	Height(mV)	Area(%)	Height(%)	$W_0$5(min)
1	14.520	180.452	2.534	10.932	0.249	1.307
2	16.880	446.854	21.603	1.304	1.749	0.260
3	18.827	7328.99	1055.07	80.743	97.980	0.807

TABLE 4:CHROMATOGRAPHIC OBSERVATIONS OF ISOLATED ALA SOLUTION.

Calculation:

Calculation of % 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.

$$\% \text{ Assay} = \frac{80.743}{100} \times \frac{0.0204}{20} \times \frac{20}{0.02037} \times 100$$

= 80.05%

Percentage purity of isolated ALA: 80.743 %

RESULT AND DISCUSSION

The isolated fraction of ALA having R_f value 0.86 when compared with standard ALA which matched with the R_f value of standard ALA. The retention time of isolated ALA and standard ALA were 18.83 and 17.981 respectively. Isolated ALA showed 80.743 % purity

CONCLUSION

It is concluded that besides fish oil, plant oil can be used as the precursor of ALA The developed method of column chromatographic isolation of ALA is simple, accurate and precise.

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