

Estimation of Fatty Acids in Corn Oil by Gas Capillary Chromatography

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Abstract

Fatty acids provide energy as well as play important role in some cellular structures like cell membrane and certain hormones. Saturated fatty acids are usually found in animal products and in some vegetable oils as well. These saturated fatty acids may be a factor in weight gain and obesity but eating them in moderate amounts may not be damaging to health of every person. Monounsaturated fatty acids can lower blood levels of low-density lipoprotein cholesterol and have potential to increase blood levels of high-density lipoprotein cholesterol and by this way plays protective role against heart disease. The omega-3 and -6 fatty acids have vital roles in many biological systems such as nervous, immune, cardiovascular, dermal and vision systems. Therefore, it is essential to optimize the instrumental conditions and column specification for the estimation of various fatty acids in the oil, which was considered in the current study using Gas Capillary Chromatography.

Keywords: Capillary, Fatty acids, Gas Chromatography, Volatile

Short running title: Estimation of FA by GC

Abbreviations: ECD, electron capture detector; FID, flame ionization detector; GC, Gas Chromatography; MS, Mass Spectrometry; PID, photoionization detector.

Introduction

Generally gas chromatography (GC) is widely used for the forensic characterization of petroleum related products such as gaseous hydrocarbons using flame ionization detector (FID), identification of major volatile hydrocarbons in the gasoline range using high resolution GC/FID, volatile aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes; BTEX) using photoionization detector (PID) or GC/MS, alkyl lead speciation and lead scavengers using electron capture detector (ECD) while oxygenated blending agents (alcohols and ethers) by two-dimensional GC/FID (www.zymaxforensics.com). Biological mixtures such as fatty acids can be separated and quantified by using gas capillary chromatography, where the capillary system

involves in splitting the sample to prevent sample overloading on the GC. A make up gas like nitrogen is usually mixed with the column effluent prior to the FID to improve response characteristics (Ettre L.S. 1985). James and Martin first demonstrated GC for separation of fatty acids of methyl esters and this technique is still popular application of GC (Rothart H.L. 1977. Gunstone F.D. 1967).

Materials and Methods

Materials

Following reagents were used in this study: methanolic NaOH (0.5 M), saturated NaCl solution (100 mL per sample), hexane or heptane (AR grade), boron trifluoride (14% in methanol).

Column specification and optimization of instrumental conditions

Fused silica was used as a material of construction in

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this column (30 m x 0.25 mm) of 0.25 μ phase thickness. Polyethylene glycol was selected as a liquid phase for this type of column and separation of FA sample. Flame ionisation detector temperature and injector temperature was 220 °C while oven temperature was set at 170 °C. The run time was 12 min and run timed event was programmed as 500 area reject at 1 min.

Methods

For a saponification process 1 gm cooking oil was added to 10 mL of 0.5M (methanolic) NaOH, in a 150 mL round bottom flask. It was refluxed until saponification was completed, i.e. when the oil globules have gone (10 min). For an esterification process after cooling, 12 mL of boron trifluoride (14% in methanol) was added without removing the condenser, and refluxed for an additional 2 min. It needs a special care in handling boron trifluoride due to its toxic and explosive nature. 2 mL of hexane was added further in a round bottle flask and refluxed again for couple of min. After its cooling, saturated NaCl solution was added until the “organic” layer reached the narrowest part of the neck of the flask, which was transferred to a small specimen tube. A small amount of anhydrous sodium sulphite was added and mixed well to clear the sample. After 50 times dilution of

the sample by hexane, it was stored at low temperature under nitrogen for applying at the appropriate time when all samples and standard to be ready for injection (Methods of analysis of A.O.A.C. 1975).

Turning off the instrument

Oven temperature was set at 80 °C while the make up gas was turned off carefully in order to activate turning off method on the computerized system, “Hewlett Packard HP series GC”. Air and hydrogen were turned off at the pressure regulator, when it became empty hence the detector was also turned off in order to release the pressure regulator. Finally, inlet pressure regulator was set at 2-4 psi.

Results and Discussion

Pressure regulator was adjusted by data processor started immediately after injection of acetone (0.4 μ L) to determine the linear velocity (l_v) from the retention time of the first peak (Eq. 1). Standard (0.4 μ L) was injected and the detector’s response to the compounds of interest was corrected by the use of area normalization. The standard mix was run again to average the calibration. The relative percentage composition of sample (0.4 μ L) was determined after its injection. GC profile for

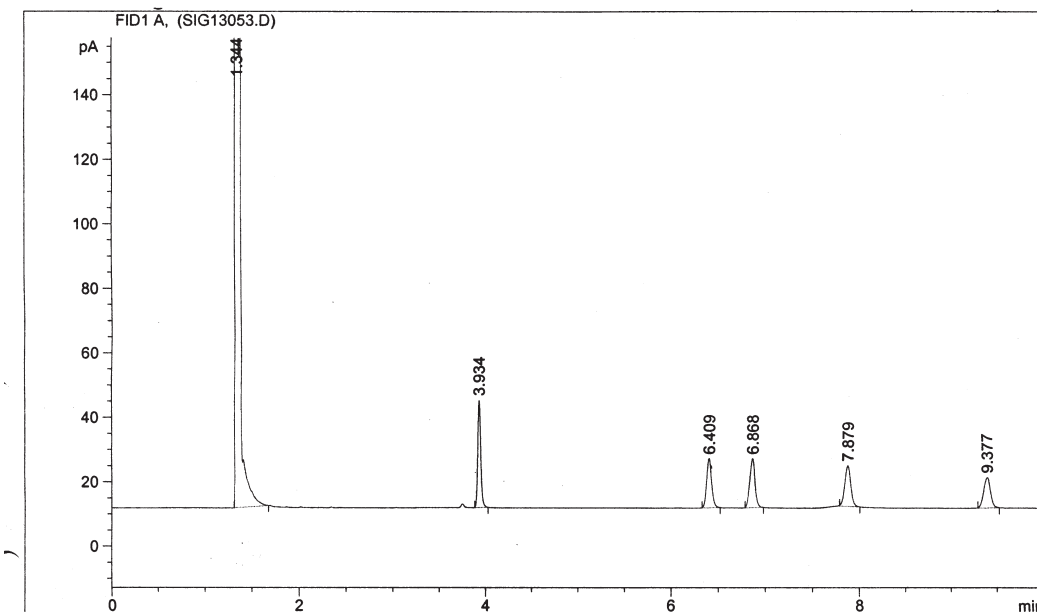


Fig 1. GC profile for standard Fame Mix (GLC-10).

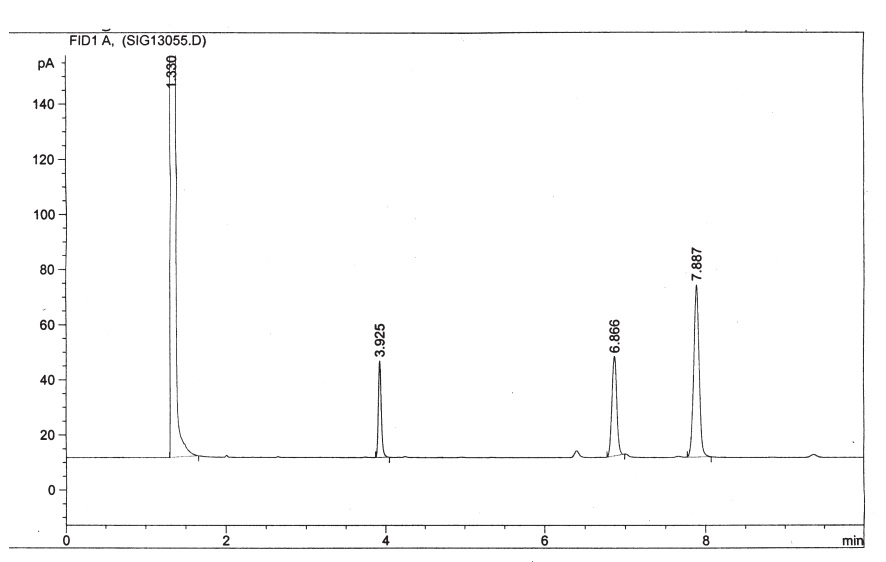


Fig 2. GC profile for corn oil.

standard Fame Mix (GLC-10) and for sample (corn oil) profile is presented in Fig. 1 and Fig. 2 respectively. The polyunsaturated to saturated ratio (P/S) for corn oil was found as follows: (1) 4.1 for unsaturated [linoleic acid / (palmitic acid + stearic acid)] to saturated ratio; (2) 6.1 for polyunsaturated [(linoleic acid + oleic acid) / (palmitic acid + stearic acid)] to saturated ratio. Regarding comparison of this finding with previous reports there are some variations in the percentage value of each fatty acid (Wills R.B., Myers P.R., Greenfield H. 1982).

$$l_v = \text{column length (cm)} / \text{retention time (sec)} \quad (1)$$

Saponification process converts the oil into a salt form of respective fatty acids by hydrolysis of triglycerides due to addition of methanolic NaOH. The advantage of esterification in the analysis of fatty acids is to achieve saponification and micelle formation. Secondly, fatty acids are converted in to the polar solute form which is better state to bind with a stationary phase and consequently results a better separation for a quantitation purpose.

There are two major types of polyunsaturated fatty acids in the diet: 1. omega-3 (alpha-linolenic acid, eicosatetraenoic acid, eicosapentaenoic acid and docosahexaenoic acid. 2. omega-6 fatty acid is linoleic acid found mostly in plant oils. The omega-3 and -6 fatty acids are converted into hormone-like substances called eicosanoids. The eicosanoids derived from omega-6 fatty acids tend to encourage inflammation, blood

vessel construction and blood clotting. By this way, they increase the risk of coronary artery disease, stroke and inflammatory conditions such as arthritis, while omega-3 fatty acids play opposite role, therefore, it is important to balance their intake in daily diet. A ratio of 1:1 is believed to be ideal.

The conclusion of this study is obvious in estimation of different fatty acids in corn oil as follows: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2) to about 13.2, 1.0, 28.2 and 57.7 % respectively.

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تقدير الأحماض الدهنية في زيت الذرة باستخدام طريقة الغاز الشعريّة الكروماتوجرافية

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الملخص

الأحماض الدهنية تمنح طاقة حيوية بالإضافة لكونها تلعب دور رئيسي في تركيب الأجزاء الخلوية مثل الغشاء البلازمي وبعض الهرمونات. وعادة ما توجد الأحماض الدهنية المشبعة في المنتجات الحيوانية وبعض الزيوت النباتية. تلعب الدهون المشبعة كعامل في زيادة الوزن والسمنة ولكن استخدامها في الغذاء بكميات معقولة قد لا يسبب ضررا للصحة لكل شخص. الأحماض الدهنية غير المشبعة - أحادية الرابطة المضاعفة - تخفض من مستوى الدهون قليلة الكثافة في الجسم والمسئولة عن نقل الكوليسترول . ولها القدرة على زيادة مستوى الدهون العالية الكثافة وبالتالي تشارك في حماية القلب من بعض الأمراض. إن الأحماض الدهنية الغير مشبعة والتي تحتوي على ثلاثة روابط مضاعفة لها دور رئيسي وهام في العديد من الأنظمة الحيوية مثل الأنظمة المناعية الأوعية الدموية، الجلدية، والنظر. لذلك كان من الأساس أهمية تحديد الظروف العملية والمعلمية لعملية تقدير العديد من هذه الأحماض الدهنية في الزيوت والتي أخذت في الاعتبار في هذه الدراسة باستخدام تقنية الغاز الشعريّة الكروماتوجرافية.