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Original Article

Synthesis and Application of 4-Acetyloxy-3-Methoxy Benzaldehyde as Chromogenic Spraying Reagent on Thin Layer Chromatography for Forensic Identification and Detection of Some Food Oils

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Abstract

Oils and fats analysis covers a major area in forensic chemistry as these are often adulterated in various ways viz., adulteration of lower grade, different origin (vegetable oil by animal fat or vice-versa) or misbranding or contra banding of products etc., the cases as above may occur frequently as oils and fats are extremely used as cooking media and also in industries like paints, varnishes, pharmaceutical industries. There may be theft cases or illegal possession related to oils and fats. In the above case the samples are frequently sent for their examinations for different purposes under Essential Commodities Act (E.C. Act).

In this study we have synthesized 4-Acetyloxy-3-methoxybenzaldehyde as spray reagent for thin layer chromatography examination of Soyabean oil, Mustard oil, Groundnut oil, Coconut oil, Rapseed oil, Linseed oil, Castor oil, and Almond oil in different solvent system. The spray reagent develops pinkish violet color.

Keywords: Oil; Fat; Fatty Acids; Thin Layer Chromatography.

Introduction

Vegetable oils are mainly constituted by triacylglycerol (95-98%) and complex mixtures of minor compounds (2-5%) of a wide range of chemical nature. These minor constituents show a broad qualitative and quantitative depending on the vegetable species from which they are obtained.

The minor components include mono- and diglycerides, free fatty acids, phosphatides (or phospholipids), sterols, protein fragments, various resinous and mucilaginous materials and oxidative products a triglyceride is a chemical compound formed from one molecule of glycerol and three fatty acids. Fatty acid chains may contain one or more double bonds at specific positions (unsaturated and polyunsaturated), or they may be fully saturated.

The physical and chemical properties of a fat depend on the composition of the fatty acid mixture. Fats from plant sources contain a higher proportion of unsaturated acids and are often liquids at room temperature due to hydrogen bonding. Fats are used in cooking because they are compounds with very high boiling points. Their high boiling points therefore make these classes of compounds ill suited for analysis by the gas chromatography. However, the glycerol ester can be chemically decomposed into partition methyl esters of each individual fatty acid. Oils and fats are important parts of human diet and more than 90 % of the world production from vegetable, animal and marine sources is used as food or as an ingredient in food products. Oils and fats are equivalent amount of sugar. Their functional and textural characteristics contribute to the flavor and palatability of natural and prepared foods.

They contain certain fatty acids, which play an important role in nutrition and are also carriers of fat-soluble vitamins. Vegetable oils has become more attractive recently because of its economic benefits as they are used as components in many manufactured products and the fact that it is made from renewable

resources. The determination of the minor components is of great importance in establishing the oil quality and their genuineness. This paper shows the applications of TLC for detection and identification of various food oils using 4-Acetyloxy-3 methoxybenzaldehyde as chromogenic detector. Fatty acids are used in cosmetics, medicines, paint industries, food industries, in the manufacture of soap as lubricants, as a fragrant material i.e. It covers almost all arena of society. Therefore they frequently encountered for forensic identification. Various oils detected previously using different locating reagents on thin layer chromatography viz. mainly sulphuric acid, anisaldehyde etc., in the present study we have synthesized new aldehyde 4-Acetyloxy-3-methoxy benzaldehyde via Acetylation of 3-Methoxy-4-hydroxybenzaldehyde, which is a white crystalline substance and forms pinkish violet color with different oils. In the present study we have used Soya bean oil, Mustard oil, Groundnut oil, Coconut oil, Rapseed oil, Linseed oil, Castor oil and Almond oil for detection and identification using this aldehyde as locating reagent on TLC plate.

Materials and Methods

Pure food oils, samples of ISI / agmark grade procured from local market of Gwalior. Methanol, ethanol, chloroform, Petroleum ether, n-butanol, glacial acetic acid, benzene, diethyl ether used were of A. R. grade, double distilled water used throughout the study.

Synthesis of 4-Acetoxy-3-methoxybenzaldehyde

Dissolve 1.5 g of 3-Methoxy-4-hydroxybenzaldehyde in 25 ml of 10% sodium hydroxide in 250 ml conical flask add 30 gm of crushed ice and 4 ml of acetic anhydride place the stop cork and shake for several times over 20 minutes of time a cloudy, milky white precipitate is formed which is filtered through Buchnar funnel and washed with ice cold water and recrystallized it from 95% ethanol which gives white crystalline needles, molecular formula C10H10O4, molecular weight 194.18.

Table 1: Rf values of different oils under different solvent system

S.No	Solvent System	Rf value of Food oils							
		Soyabean Oil	Mustard Oil	Groundnut Oil	Coconut Oil	Rapseed Oil	Linseed Oil	Castor Oil	Almond Oil
1.	Petroleum: Diethylether: Acetic acid (9:10:1)	0.1, 0.25, 0.3, 0.5 ,0.7	0.2,0.35, 0.5,0.65	0.35,0.5,0.7, 0.8, 0.9	0.15, 0.3,0.65,0.7	0.1, 0.35 0.5,0.7, 0.9	0.35, 0.6, 0.8	0.2,0.3 5,0.5,0 .6,0.7, 0.9	0.2,0.25, 0.3,0.5,0 .65,0.7, 0.9
2.	Hexane: Ether(1:1)	0.1, 0.3, 0.5, 0.65, 0.7, 0.85	0.15,0.2, 0.4,0.5,0 .55,0.6, 0.8	0.15,0.2,0.3, 0.4,0.55,0.6, 0.75,0.9	0.3,0.45, 0.5,0.7	0.2,0.3,0.35 ,0.45,0.6,0. 75, 0.8,0.9	0.4,0.55,0.6 ,0.7,0.9	0.2,0.2 5,0.4, 0.5,0.5 5,0.6, 0.7,0.9	0.25,0.3, 0.5,0.6,0 .75,0.8, 0.9
3.	n-Butanol: Acetone:Etha nol:Water (60:20:20:1.5)	0.65,0. 8,0.9	Streak b/w 0.6-0.9	0.85	0.9	Streak b/w 0.7-1	streak at 0.7	0.65, 0.8	0.5,0.6, 0.8
4.	Benzene: Acetic acid(100:1)	0.6,0.7 5,0.8	0.7,0.85, 0.9	0.8	0.5,0.7, 0.8	0.75,0.8	0.65,0.80.9	0.7,0.8 5,0.9	0.5,0.75, 0.9
5.	Hexane:Aceto ne(6:4)	0.8,09	0.9	0.9	0.85,0.9	0.9	0.7,0.85	0.8	0.75,0.8 5,0.9
6.	Toluene:Chol orform (3:1)	0.65,0. 8,0.9	0.2,0.5,0 .8,0.9	0.5,0.7,0.85, 0.9	0.6,0.75,0.9	0.2,0.65,0.8 ,0.9	0.7,0.85,0.9	0.2,0.6 ,0.75,0 .8,0.9	0.4,0.7, 0.8
7.	Heptane (100%)	0.5,0.6 ,0.9	0.4,0.5, 0.8	0.3,0.4,0.6	0.5,0.6	0.3,0.4, 0.5,0.8, 0.9	0.6,0.9	0.2,0.4 ,0.5,0. 6,0.8, 0.9	0.7
8.	n-Butanol: Acetic acid: Water (60:15:25)	ND*	ND*	ND*	ND*	ND*	ND*	ND*	ND*
9.	Methylene chloride:Ether :Methanol: Water (77:15:8:1.2)	0.5,0.9	0.9	0.7,0.75, 0.9	0.8,0.9	0.7	0.5,0.9	0.9	0.6,0.8
10.	Dichloro ethane: Methanol:Wat er (95:5:0.2)	0.7,0.9	0.6,0.9	0.4,0.5,0.7	0.9	0.8	0.5,0.7, 0.8	0.7,0.9	0.6,0.75

*No Development

4-Acetoxy -3-methoxybenzaldehyde Preparation of reagents

(A) 0.328 gm of 4-Acetoxy -3-methoxybenzaldehyde in 100ml of absolute alcohol.

(B) 50% aqueous sulphuric acid.

Thin Layer Chromatography Analysis

A Standard glass TLC plates was coated with slurry of silica gel G water to a uniform thickness of 0.25 mm. The plate was activated by heating an oven at 110^{0}C for about one hour. Aliquots of different oils were spotted on to the plate, using fine capillary tubes TLC plate and allowed to dry for a few minutes. A TLC developing chamber containing the solvent system was properly saturated using filter paper strips, and the spotted TLC plate was placed vertically in it, and the chamber was covered with a lid. Separation of the samples was achieved after running the solvent system petroleum ether: diethyl ether: acetic acid (90:20:1ml) for a distance of 10 cm from the point of spotting.

The TLC plate was then removed from the chamber and dried at room temperature. The plate was removed from the chamber dried in air sprayed by reagent (a) followed by reagent (b) and heated in oven for one hour then colored spots appeared against the white background. The Rf values of different oils under different solvent systems are given in the Table1.

Results & Discussion

TLC analysis was carried out to study the difference in the constituent profiles of food oils. A marginal difference in constituent profiles of these samples was observed, which is because plants have their own distinctive chemical component profiles. TLC analysis method for the analysis of above mentioned test oils revealed several spots, which could be separated by using various solvent systems *(Table1),* and visualization was done by spraying 4-Acetoxy-3-methoxybenzaldehyde reagent. Some solvent systems used for the TLC analysis of test oils have revealed several spots after separating in solvent systems. In the present work, ten solvent systems were tried (Table 1), out of which eight solvent systems Hexane: ether (1:1), n-butanol: acetone: ethanol: water (60:20:20:1.5), Benzene: acetic acid (100:1), Hexane: acetone (6:4), Toluene: cholorform (3:1), Heptane (10%), Methylenechloride: ether: methanol: water (77:15:8:1.2), Dichloroethane: methanol: water (95:5:0.2) gave useful results, but n-butanol: aceticacid: water (60:15:25) solvent system shows no development and *Petroleum: diethyl ether: acetic acid (9:10:1)* was the only solvent system that provided satisfactory separation.

In this study the used locating reagent develops bright pinkish violet color which distinctively identifiable. This reagent is quiet sensitive and develops color without hindrance and is highly cost effective and identifies samples in microgram quantities. In the study various solvent systems gave distinctive separations of constituents of food oils.

Conclusions

In this study, *Petroleum: diethyl ether: acetic acid (9:10:1)* have been found to be the best solvent system for the proper extraction of the selected samples. Ten solvent systems were tried, and only this could produce fruitful and reproducible results. The constituents of the oils of the different plant species undertaken in the present study can be separated and differentiated for the purpose of identification by this method. Spots were visualized best by using 4-Acetoxy -3-methoxy benzaldehyde as spray reagent. The use of 4-Acetoxy-3-methoxybenzaldehyde in detection and identification of food oil is quiet useful, less time consuming, sensitive and gives accurate results. Therefore it can be used in routine forensic examination of oil and fats.

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