

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) SEPARATION OF SOME FATTY ACIDS AS NAPHTHYL METHYL DERIVATIVES USING RESIN SUPPORTED 1-NAPHTHALENE METHANOL AS THE DERIVATIZATION REAGENT

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ABSTRACT

Cation exchange resin in the sulphonyl chloride form was prepared by reacting cation exchangers in the sodium form with calculated amount of phosphorus pentachloride by refluxing. The sulphonyl function on the resin serves as an anchor through which naphthalene methanol moiety was bonded to the immobile resin backbone. The resin supported 1-naphthalene methanol was subsequently employed to derivatize some fatty acids. The reagent imparted fluorescence property on the final derivatives. These derivatives were obtained in pure form and simply isolated by filtration.

The derivatives absorbed UV-radiation strongly at 254nm and direct HPLC analysis was found possible without interference from excess reagent; This indicated that the resin supported 1-naphthalene methanol may be employed for heterogeneous on-line pre-or post column derivatization of fatty acids.

Keywords: Resin, Moiety, Derivative, Fluorescence, Heterogeneous

INTRODUCTION

In recent years, fatty acids have been of considerable importance; for instance, some C20 acids are known precursors of the biologically important prostaglandins (Cooper & Ander, 1994). and are constituents of some drugs like folic acids and indomethacin which are employed for treatment of megaloblastic anaemia and inflammatory joint disease respectively (Sackhein and Lehman, 1981). Similarly, the analysis of some short chain fatty acids have found tremendous importance in the study of genetic disease and of the physiology and taxonomy of micro-organisation (Farinotti et. al., 1983).

The ability to investigate this functionality is very important and several techniques to detect or confirm their presence are needed. The initial approach for their determination was gas-liquid chromatography (GLC), either by direct injection (Burchfield and Stoops, 1962). or after derivatization (Farinotti et. al., 1983) In liquid chromatography (LC), compounds can be detected by using UV, fluorescence or electrochemical detection. For

compounds that are transparent to these detectors, labelling with UV adsorbents, fluorescent (Farinotti et. al., 1983), (Lam and Grushka, 1978), (Adewuyi et. al., 2001) or electro-active nuclei (Farinotti et. al., 1983) before or after chromatography may provide a solution.

In converting fatty acids to UV – absorbing or fluorescent derivatives for the purpose of HPLC analysis, solution reagents like phenacyl bromide and naphthacyl bromide (Allehmarks and Bertilsson 1988, Cooper and Anders, 1974) have been used. The major draw back of this reagent is that they constitute excess reagents which may interfere with the detection of the analyte. Introduction of steps to remove excess reagents often make the derivatization process to be more tedious and more prone to error. These and other problems can be circumvented by employing chemically reactive insoluble resin bound reagents. Though, these supported reagents have found wider application in organic synthesis (Rubinstein and Patchonik 1975, Hodge and Sherrington 1980, Mathur and Narang 1980) they are however, of limited

use in HPLC derivatization of fatty acids.

Where they are employed, the reagents used are not readily accessible because their preparation requires special polymeric supports, the reagents have therefore, not found routine application in HPLC derivatization of fatty acids supported reagents in which the reagents are ironically bound to insoluble resin support. These are to be preferred since readily available ion-exchange resins could be easily employed in their preparation and they would have a greater shelf-life.

The present communication therefore, is on the use of resin supported 1-naphthalene methanol as a reagent for heterogeneous derivatization of fatty acids for the purpose of HPLC with fluorescence or UV detection.

MATERIALS AND METHODS

Chemicals

The following chemicals were obtained from BDH (Poole, England). Amberlite[®] 1R^A 120 ion-exchange resin (Na⁺ form), phosphorus pentachloride, potassium hydroxide pellets, silver nitrate, AR: acetic acid, valeric acid, hexanoic acid, nonanoic acid, palmitic acid and ethanol were used.

Instrumentation

Infrared spectroscopic identification was obtained using perkin-Elmner 599B infrared spectrophotometer. by KBr disc.

HPLC was performed on a Philips PU 4110 absorbance detector set at 254nm. The column specification was Bondaclore 10 C₁₈ (3.9mm x 300mm). The recording temperature was ambient; methanol: water (1:1) served as the eluent while the flow rate was 1.3ml/min.

Preparation of sulphonyl chloride bond resin

Resin-bound sulphonyl chloride was prepared using the method of Adewuyi

(1997) by suspending air-dried cation-exchange resin (10.0g) in the sodium form (capacity, 3.45 meq/1g) in Nitrobenzene (50 ml) for 1h. After which the suspension was cooled to 0°C in an ice-water bath and filtered by suction. The swelled resin was treated with phosphorus pentachloride (7.2g) and the mixture was refluxed on an oil bath at 110°C for 4h. After reflux, the mixture was allowed to cool at room temperature and washed by suction with distilled water, and finally washed with anhydrous acetone (50 ml). The product was dried under reduced pressure.

Preparation of 1-naphthalene methanol

The method of Adewuyi (1997) was used to prepared 1-naphthalene methanol. 1-chloromethyl naphthalene (15.0g) (11, 6) was dissolved in 50ml acetone. The solution was added gently to a solution of silver nitrate (14.0g) in 80ml acetone containing 20ml of distilled water. The solution of 1-chloromethyl naphthalene was then treated with the solution of silver nitrate. Silver chloride was immediately precipitated as light yellowish solid. The solid was filtered off and the filtrate treated gently with equal volume of distilled water to precipitate 1-naphthalene methanol as a whitish solid. The solid was filtered and dried in air.

(Theoretical yield = 13.4 g actual yield = 6 g, yield = 45%)

Preparation of 1-naphthalene methanol bond resin

1-naphthalene methanol bond resin was prepared according to the method used by Adewuyi (1997). Resin sulphonyl chloride (5.0g) was packed in a quick fit boiling tube and 10ml dimethyl formamide was added to completely wet and swelled the resin. The mixture was left for 45 minutes before it was treated with a solution of 1-naphthalene methanol (10.0g) dissolved in 15ml dimethyl formamide. 20ml of triethyl amine was later added and the mixture refluxed for 1.5h. The mixture was allowed to cool to room temperature after which it was filtered by suction to obtain the resin supported 1-naphthalene methanol which was washed several times with acetone to eliminate the excess unreacted 1-naphthalene methanol. The resin supported 1-naphthalene methanol (dark brown, 4.08 g) was allowed to dry in air.

Reaction of fatty acids with 1-naphthalene methanol bond resin.

To a solution of 100µl of acetic-, or valeric-, or hexanoic-, or octanoic- or nonanoic- or capric acids in 1.0ml of solution of potassium hydroxide in 90% aqueous ethanol was added 1.0g of resin tagged 1-naphthalene methanol. After warming in a water bath (80°C) for 15 min, the solution was examined under UV light and later analyzed by HPLC.

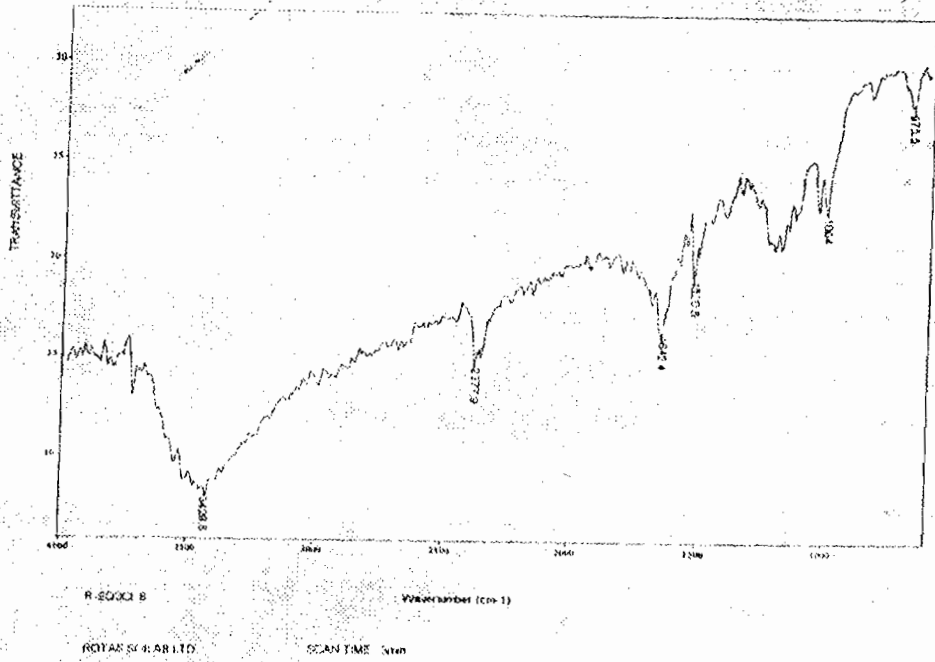


FIG 1 : Infra-red spectrum of resin in sulfonyl chloride form

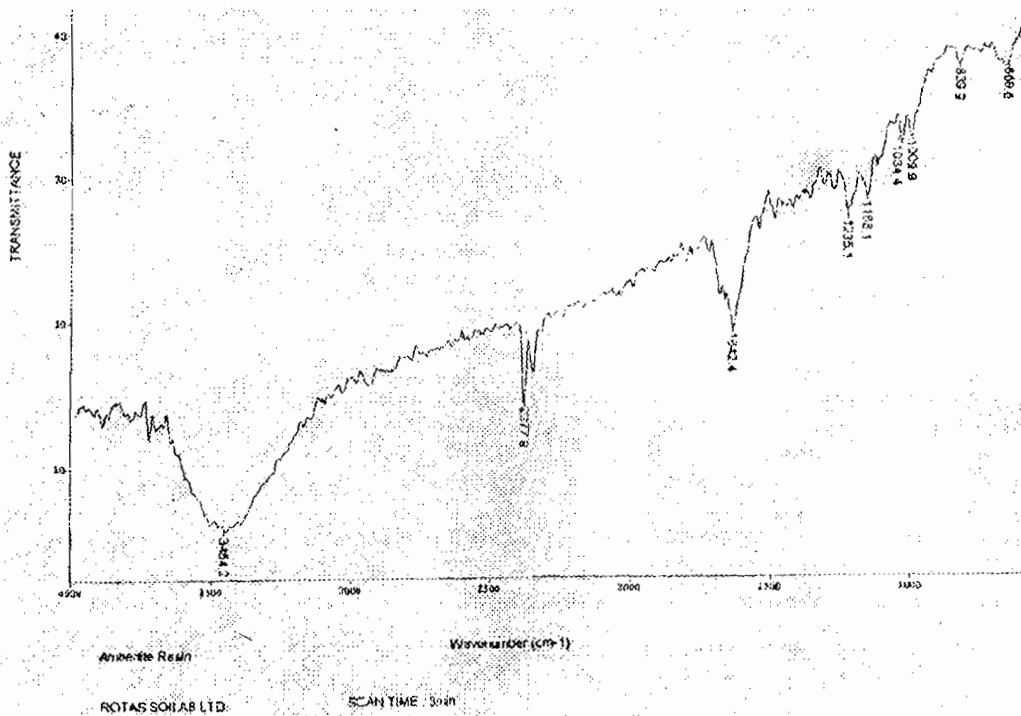


FIG 2 Infrared spectrum of amberlite resin in sulfonic acid form

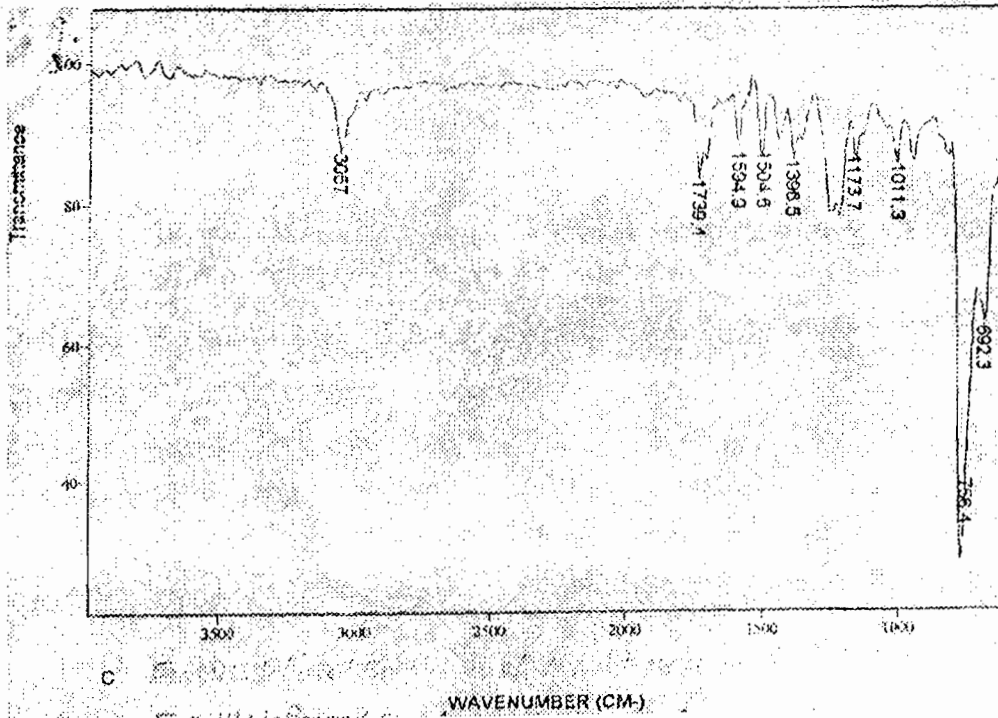


FIG. 3 : Infrared spectrum of Naphthalene methanol

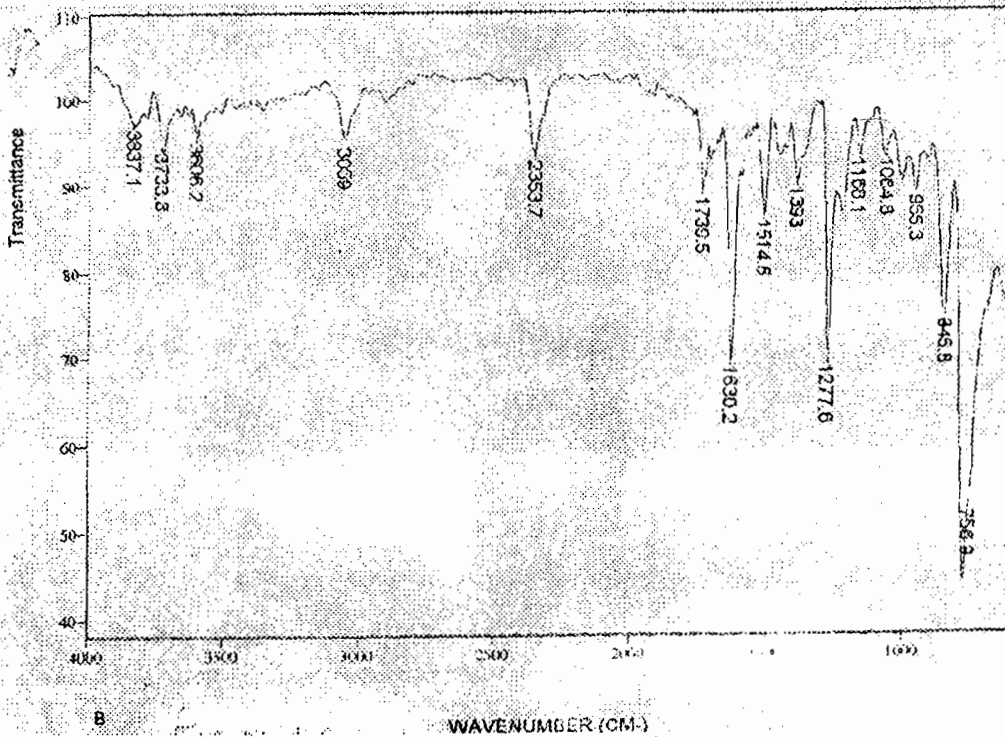


FIG. 4: Infrared spectrum of Resin Supported Naphthalene methanol

RESULTS AND DISCUSSION

Formation of the sulphonyl form of the resin was confirmed from its infrared spectrum (fig. 1) when compared with the infrared spectrum of the amberlite cation exchange resin in the sulphonic acid form (fig 2).

(Mathur, Narang and Williams, 1980) have reported that the infrared spectra of functional groups anchored on resins do not differ appreciably from those in small molecules. In the spectra of the two forms of the resin, the most significant absorptions in the amberlite cation exchanger occurred at 1168.1cm^{-1} , 1235.1cm^{-1} and 1009.9cm^{-1} while, the characteristic absorption for the resin in the sulphonyl chloride forms were observed at 1004cm^{-1} , 1168cm^{-1} and 1520.8cm^{-1} respectively. These absorptions could be interpreted as follows: The symmetric $\text{S} = \text{O}$ stretching frequency for sulphonic acids appearing at 1168.1cm^{-1} was common to the two forms of the resin. But the two characteristic absorptions which showed the dissimilarity of the two forms of the resin were those found in the cation exchanger spectrum at 1235.1cm^{-1} region and the one occurring in the resin sulphonyl chloride spectrum at 1520.8cm^{-1} region respectively. Literature reveals (Sliverstein et. al., 1975) that the sulphonic acid hydrate readily gives the type of band at 1235.1cm^{-1} resulting from the formation of hydromium sulphate. This band was absent in the resin sulphonyl chloride spectrum. However, the band that was observed 1520.8cm^{-1} in the resin sulphonyl chloride spectrum was also absent in the spectrum of the cation exchange resin and it has been shown that sulphonyl chloride absorbs strongly in this region (Henry et. al., 1971).

The increase in frequency when compared to the sulphone absorption resulted from the electronegativity of the chloride ion. These characteristic absorptions are suggestive of formation of the resin in the sulphonyl chloride form. A further chemical confirmatory test was performed to test for the presence of chloride ion on the resin sulphonyl chloride form. When silver nitrate solution (5ml) and dilute nitric acid (2.5ml) were added to about 2.0g of the resin sulphonyl chloride a white precipitate was formed, confirming the presence of a chloride ion. When a similar test was performed on the amberlite cation

exchange resin in the sulphonic acid form, no precipitate was observed, an indication that chloride ion was absent in this form of the resin.

In the 1-naphthalene methanol spectrum (fig 3) characteristic absorptions were observed at the region 756.4cm^{-1} and 692.3cm^{-1} . These absorptions could be attributed to out of plane aromatic C - H bend and ring C...C bend. The band which was due to C...O stretching vibration in aromatic alcohol was observed at 10.113cm^{-1} while the absorption for O...C...C asymmetric stretching vibration occurred at 1173.7cm^{-1} . The characteristic band for hydrogen bond O-H...O-H stretch which is normally observed for the spectra of alcohols also occurred at 3057cm^{-1} . Similarly, the band that was due to ring stretch in aromatic compound was similarly observed at 1594.9cm^{-1} . These absorptions are in line with literature predictions for such aromatic alcohols (Sliverstein et. al., 1974). Hence, these characteristic absorptions are suggestive of the formation for 1-naphthalene methanol.

Formation of the resin supported Naphthalene methanol was confirmed from its infrared spectrum (fig. 4). In the spectrum, characteristic absorptions indicating the presence of sulphonium group were observed at 1064.8cm^{-1} , 1168.1cm^{-1} and 1514.6cm^{-1} respectively. The absorption at 1168.1cm^{-1} indicated the symmetric $\text{S} = \text{O}$ stretching frequency for sulphonium group. The absorptions in region 1064.8cm^{-1} and 1514.6cm^{-1} were responsible for the $\text{S} - \text{O} - \text{C}$ stretching vibration. These absorptions are in accord with literature prediction Sliverstem et.al., and Morril (1974). Also, literature further reveals (Sliverstein et. al., 1974) that the strong absorptions that were observed in region 756.9cm^{-1} and 845.5cm^{-1} were due to C-H out of plane bending vibration for four hydrogen of a substituted Naphthalene and C-H out of plane bending vibration for two adjacent hydrogen of a substituted Naphthalene respectively. The absorptions 1630cm^{-1} to 2353.7cm^{-1} were due to overtone or combination bands (Sliverstein et. al., 1974). The appearance of sharp absorptions at 3606.2cm^{-1} , 3733.9cm^{-1} and 3837.1cm^{-1} indicated the spectral bands due to the CH_2 group in epoxy resin (Sliverstein et. al., 1974). These particular absorptions were absent in the unanchored 1-naphthalene methanol moiety. These characteristic absorptions are suggestive

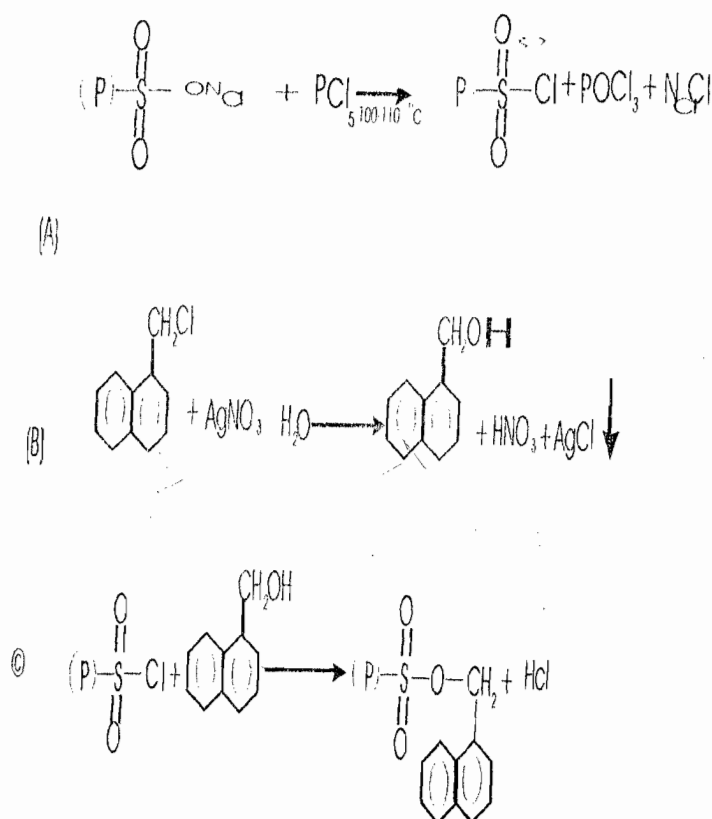


Fig. 5 (a-c) Illustrating the reaction sequences leading to the formation of 1-naphthalene methanol bound resin.

of the formation of the resin supported 1-naphthalene methanol.

Derivatisation studies

The most suitable derivatisation reagent for TLC or HPLC with UV or fluorescence detection must have certain characteristics. Its UV or fluorescence characteristic should be completely different from that of the derivative to prevent excess reagent from interfering with detection of the derivative. But, if the reagent and the derivative possess similar spectral characteristic, their chromatographic behaviour should be widely different to enable easy separation of the excess reagent from the derivative, if such separation cannot be achieved by solvent extraction (Idowu and Adewuyi, 1997).

Furthermore, the derivatisation reagent should react readily with the analyte without complicating side reactions, and the derivative that result from such reaction must be stable. Also, it should be possible to conduct the derivatisation reaction in variety of solvents and solvent combinations that are likely to be encountered during the intended chromatographic application. The reagent

must be readily available and cheap so that it can be easily used for routine investigation of the analyte.

Derivatisation reagent

The sulphonyl chloride bound resin was prepared and chosen for the attachment of the fluorescent moiety (1-naphthalene methanol), because of the greater nucleophilicity of sulphur group and the relative physical stability of the polystyrene backbone. The reaction sequences leading to the formation of 1-naphthalene methanol bound resin are shown by equations in figures (5a-c). The general solid face reaction of short chain carboxylic acids with the granular 1-naphthalene methanol bound resin is shown by the equation in figure 7. The resulting derivative is naphthyl-methyl derivative.

Chromatographic separation of fatty acids derivatives

The UV detector is the most convenient and sensitive detector for high performance liquid chromatography when the compounds under probe have a reasonable extinction coefficient at the detecting wave length

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(Henry, et. al., 1971). Figure 6 shows a separation of six fatty acids using UV detection. Since these derivatives have incorporated into their structures a naphthyl methyl group see (fig. 7) for illustration, therefore, they are expected to possess strong UV absorption, at 254nm that makes the UV detector preferred for these compounds.

Naphthyl-methyl derivative

The preparation of UV-absorbing derivations is a powerful tool which will allow the chromatographer, to take opportunity of the convenience and sensitivity of the UV-detector for non-absorbing or weakly absorbing fatty acids. When selecting a derivative it is pertinent to consider such factors as speed,

completeness of reaction and specificity for functional group so that the reaction can be controlled and the reaction products identified (Henry, et. al., 1971)

It is of course, important that the derivative absorbs strongly at the detection wavelength. A further consideration, should be a knowledge of how to easily chromatograph the derivative once it is formed. It is usually sufficient in this respect, to select derivatives which have solubility characteristics that are compatible with efficient, convenient chromatographic systems. Thus the incorporation of the naphthyl methyl group into the final derivatives of the fatty acids in our work had enhanced their sensitivity to the UV detector.

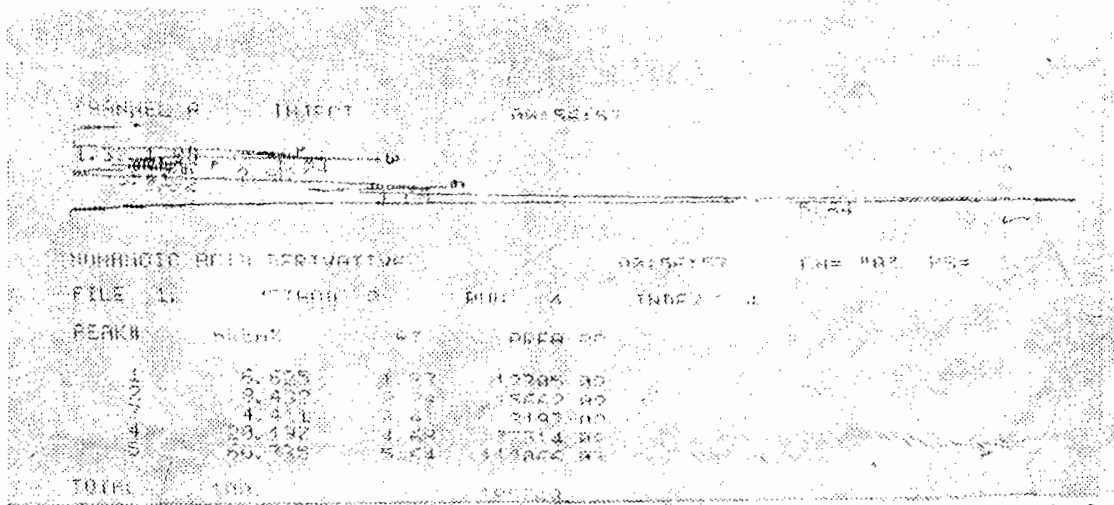


FIG. 6. Chromatogram of Naphthyl-methyl derivatives of 1. acetic- 2. valeric. 3. hexanoic- 4. octanoic- 5. nonanoic- and 6. capric acids. Column: Bondacelone 10C₁₈ i.d. 300mm x 3.9mm mobile phase: methanol: water (50%:50%) Flow rate: 1.3ml/min wavelength 254nm Temp. ambient Detector: Philips PU 4110

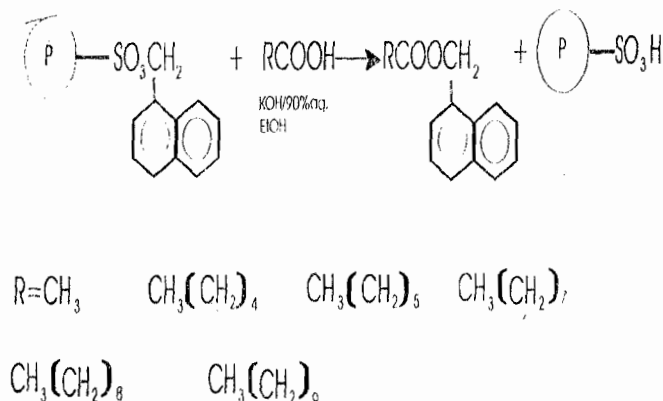


Fig. 7 An Illustration of solid-phase derivatisation reaction of fatty acids using 1-naphthalene-methanol bound resin as the derivatisation reagent.

Figure 6 shows the UV chromatographic separation of the Naphthyl methyl derivatives on a HPLC system using UV detection. Good chromatographic properties of the Naphthyl methylester were observed with methanol-water serving as the eluent. The derivatives elute in a "normal phase" order of decreasing chain length or increasing polarity. No attempts here made to optimize the system further for this separation. An impurity peak was observed in the chromatogram but it was not crucial for this study. The separation of the derivatives is comparable to that reported by (Durst et. al., 1975) though α , β dibromoacetophenone was employed as against 1-naphthalene methanol bound resin which we used.

Moreover attempts were made by the workers to derivatize acetic, propionic-, butyric- and heptanoic acids, while no attempt were made to derivatize and separate hexanoic-, nonanoic, and capric acids (Durst et. al., 1975).

Also, (Durst et. al., 1975) separated some fatty acids as methyl ester derivatives using 50 cmx2.1mm.i.d corasil II column coated with 0.8% AgNO₃/1.75% ethylene glycol stationary phase using 50% n-heptane /50 n-hexane as a mobile phase and a RI detection. Even though separation was accomplished, however, such a column is not broadly applicable to a system containing water due to its quick degradation.

The present work is an improvement over the previously conducted studies due to the ease of derivative formation, simple isolation and simple chromatographic separation. More over the development and utilization of a solid-phase reagent in which Naphthalene methanol is bound to a cation exchange resin for subsequent derivatization of fatty acids is a novel approach to derivatization studies. Thus this method of derivatization can be adapted for possible use in pre-or post column routine derivatization of fatty acids in some biological materials.

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