HPLC Determination of Monocarboxylic Acid or Mercaptans by UV-Labelling with 1-(Chloromethyl)naphtharene

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ABSTRACT

A new high performance liquid chromatographic determination of monocarboxylic acids or mercaptans at μM levels is presented. In the technique, monocarboxylic acids or mercaptans are derivatized to their 1-naphthylmethyl esters or sulfides by using 1-(chloromethyl)naphtharene as the labelling reagent. The resulting 1-naphthylmethyl derivatives are determined by high performance liquid chromatography with ultraviolet absorption detection, because the derivatives possess high ultraviolet absorption.

Key Words: High Performance Liquid Chromatography, Determination with Labelling, Monocarboxylic Acids and Mercaptans, 1-(Chloromethyl)naphtharene

Introduction

By the introduction of ion chromatography [1,2], analyses using high performance liquid chromatography (HPLC) have been widely extended, because of its unmatched ability to determine trace inorganic anions [3]. Its unmatched ability is based on the good separation of the anions by the column packed with ion exchange resin and on their highly sensitive and selective detection by electron conductivity detector. However, it is difficult to determine trace amounts of organic anions by ion chromatography because of their low electric conductivities. In the HPLC determination of organic anions, a labelling technique has usually been used for enhancement of the sensitivity of ultraviolet and fluorescence detection, and various labelling reagents have been developed [4, 5]. For carboxylic acids, frequently used were O-(*p*-nitrobenzyl)-N,N′-diisopropylisourea [6], tolyltriazene derivatives (such as 1-benzyl- [7] and 1-*p*-nitrobenzyl-3-*p*-tolyltriazene [8]), sulfonate esters (such as *p*-nitrobenzyl, 3,5-dinitrobenzyl, 2-(1-naphthyl)ethyl and 2-(phthalimino)ethyl *p*-toluenesulfonates [9], and *p*-bromophenacyl trifluoromethanesulfonate [10] and halomethyl compounds [such as substituted phenacyl bromide (*p*-bromo- and *m*-methoxyphenacyl bromide) [11–13], 9-chloromethylanthracene [14], N-chloromethyl-4-substituted phthalimide [15,16], 7-acetoxy- and 7-methoxy-4-bromomethylcoumarins [17], and 3-bromomethyl-6, 7-dimethoxy-1-methyl-2(*1H*)-quinoxalinone [18]]. On the other hand, the labelling of mercapto groups is carried out with N-(9-acridinyl)maleimide [19], N-[*p*-(2-benzimidazolyl) phenyl] maleimide [20], 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole [21], and 9-chloromethylanthracene [22]. To the author's knowledge, however, 1-(chloromethyl)naphtharene has never been used for the labelling of carboxylic acids and mercaptans for HPLC determination.

In this work, studied has been a new method for the determination of monocarboxylic acids or mercaptans with labelling with 1-(chloromethyl)naphtharene. In the technique, monocarboxylic acids or mercaptans are labelled with the above reagents to their 1-naphthylmethyl esters or 1-naphthylmethyl sulfides, respectively, which are subsequently determined by HPLC with UV detection. The labelling reactions are given as follows:

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$$\begin{matrix} RCOOH \\ \text{or} \\ RSH \end{matrix} \quad + NpCH_2Cl \xrightarrow[\text{(18-crown-6)}]{K_2CO_3} \quad \begin{matrix} RCOOCH_2Np \\ \text{or} \\ RSCH_2Np \end{matrix}$$

Np = 1-naphthyl moiety

Scheme 1

It seems that trace amounts of monocarboxylic acids or mercaptans can be determined by the method, because naphthyl moiety possesses high UV absorption. Furtheremore, it is expected that fluorescent detector responds to the labelling derivatives of carboxylic acids or mercaptans, because naphtharene fluoresces.

Experimental

Apparatus.

The HPLC system comprised a Model LC-6A pomp (Shimadzu, Kyoto, Japan), a Model 7125 syringe loading sample injector (Rheodyne, Cotati, CA, U.S.A.) and a Model SPD-6A variable-wavelength UV absorption detector. The separation column, YMC A-302 ODS (15 cm × 4.6 mm I.D., particle size 5 μm) was obtained from Yamamura Chemical Laboratories (kyoto, Japan). The mobile phase was acetonitrile at a constant flow rate of 0.5 mL/min. A Shimadzu Chromatopac C-R6A data processor was used as the detector and integrator.

Reagents.

Analytical reagent grade 1-(chloromethyl)-naphtharene and 18-crown-6 were obtained from Tokyo Kasei (Tokyo, Japan) and Aldrich (Milwaukee, WI, U.S.A.), respectively. Monocarboxylic acids were also obtained from Wako (Osaka, Japan), except for oleic acid which was from Tokyo Kasei. Mercaptans were obtained from Tokyo Kasei, except for *n*-decyl mercaptan which was from Wako. Distilled acetonitrile was used for reaction solvent and it was also used for mobile phase after filtration with a Millipore FH-0.5 μm membrane filter (Bedford, MA, U.S.A.). Analytical reagent grade 9-phenylanthracene and potassium carbonate were obtained from Tokyo Kasei and Wako, respectively.

Procedure.

The recommended procedure for labelling of monocarboxylic acids or mercaptans was as follows. A brown test tube with a screw cap (*ca.* 10 mL) was used as the reaction vessel in order to protect the content from the light. To 1.00 mL of a reference standard solution of monocarboxylic acids or mercaptans was added a solution (1.50 mL) containing 1-(chloromethyl)-naphtharene and 9-phenylanthracene as the reagent and internal standard, respectively. This solution also contains 18-crown-6 as the catalyst in the case of the labelling of monocarboxylic acids, whereas the solution does not contain 18-

Table I Optimum Labelling Reaction Conditions and Calibration Data

	capric acid	*n*-octyl mercaptan
optimum labelling conditions		
reaction temperature	65 °C	65 °C
reaction time	60 min	20 min
concentration of 1-(chloromethyl)naphtharene	10 mM	20 mM
concentration of 18-crown-6	2 mM	not used
concentration of 9-phenylanthracene[1)]	0.07 mM	0.03 mM
correlation coefficient of calibration curve	0.9986	0.9996

1) 9-phenylanthracene is the internal standard.

crown-6 in that of mercaptans. The concentrations of the reagent, catalyst and internal standard in the solution were dependent on the analytes (*i.e.*, monocarboxylic acids and mercaptans) as given in **Table I**. Then a small amount (ca. 30 mg) of anhydrous potassium carbonate was added, and the mixture was stirred at a fixed temperature for a fixed time. The suitable reaction temperatures and reaction times were also shown in **Table I**. After the reaction period, the reacted solution was filtered with a Minisart SRP 15 disposable filter holder in which 0.45 μm pore size hydrophobic membrane filter was fitted (Sartorius, Göttingen, F.R.G.). An aliquot (10 μL) of the filtrate was injected into the high performance liquid chromatograph. From the chromatogram, the resulting derivatives were determined by the internal standard method as usual.

Results and Discussion

Optimum conditions.

Capric acid and *n*-octyl mercaptan were selected as the model monocarboxylic acid and mercaptan, respectively, and the optimum detection conditions and labelling reaction conditions were tested for the acid and mercaptan.

The reacted solution of capric acid (10 μM) or *n*-octyl mercaptan (20 μM) was detected at different wavelength, in order to determine the wavelength corresponding to the absorption maximum of the derivative of each analyte. The results are given in **Figure 1**. The wavelengths which give the maximum peak areas of the derivatives are 221 nm for capric acid and 224 nm for *n*-octyl mercaptan. In this work, the detection was performed at 221 nm in both cases, because the peak area from *n*-octyl mercaptan detected at 221 nm is about the same as that detected at 224 nm. The ordinate of **Figure 1** is shown by assigning the maximum peak area of the derivative of capric acid or *n*-octyl mercaptan as 100.

Next, investigated were the labelling reaction conditions (*i.e.*, reaction temperature, reaction time and the concentrations of 1-(chloromethyl) naphtharene and 18-crown-6) for the reference standard solution containing capric acid (50 μM) or *n*-octyl mercaptan (20 μM). **Figure 2** shows the effect of the reaction time on the labelling of capric acid or *n*-octyl mercaptan. In the Figure, the peak area on the ordinate is exhibited by assigning the maximum peak area of the derivative as 100. The effect of reaction time on the labelling was tested by labelling capric acid or *n*-octyl mercaptan in the presence and absence of 18-crown-6. From the results, it is seen that use of 18-crown-6 shortens the reaction time for capric acid, while the effect of reaction time on the labelling of *n*-octyl mercaptan was independent on the use of 18-crown-6. **Figure 2** shows the effects obtained when the labelling of capric acid was carried out in both the presence and absence of 18-crown-6 and when that of *n*-octyl mercaptan was in only the absence of 18-crown-6. In further study, 18-crown-6 was used for the labelling of mono-

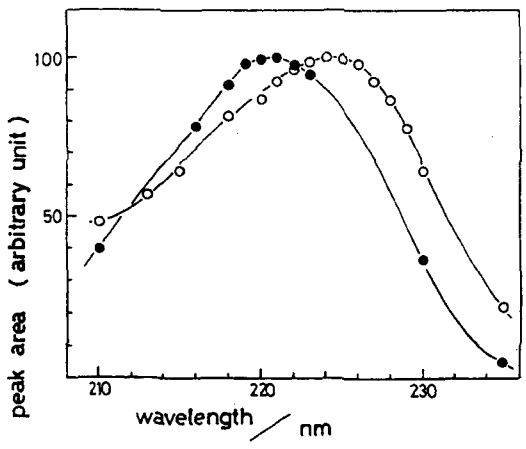

Figure 1 Effect of wavelength on the peak area of the derivatives of capric acid (●) and *n*-octyl mercaptan (○). The peak area on the ordinate is shown by assigning the maximum peak area of each derivative as 100.

carboxylic acid and not used for that of mercaptan. From the result given in **Figure 2**, the labelling reactions of capric acid (in the case using 18-crown-6) and *n*-octyl mercaptan complete within 50 and 10 min; reaction times were fixed at 60 and 20 min, respectively. By the similar tests for other parameter mention above, the optimum labelling reaction conditions were fixed as shown in **Table I**. **Figure 3** shows the typical chromatograms obtained by labelling capric acid and *n*-octyl mercaptan under the optimum conditions.

Analytical calibration and chromatograms

After the optimum labelling reaction conditions had been established, ten differnet concentrations of reference standard solutions containing 5–50 μM capric acid or *n*-octyl mercaptan were evaluated to examine the quantitative applicability of the method to the determination of monocarboxylic acids and mercaptans at μM levels. Each calibration curve was constructed by plotting the peak area ratio *versus* the analyte concentration in the solution. **Table I** also gives the results as the correlation coefficients which are nearly equal to 1 and indicates good applicability of the method to the determination.

Figure 4 shows the chromatograms obtained for determining the mixtures of monocarboxylic acids and mercaptans by absolute calibration method instead of internal standard method. Good separations were observed for mercaptans (C_6–C_{16}: even carbon number) and for saturated monocarboxylic acids (C_{10}–C_{18}: even carbon number). The addition of unsaturated monocarboxylic acids to the saturated monocarboxylic acids complicates the separation. In this work, investigated was the separation of the above five saturated acids and three unsaturated acids, the carbon number of which is 18; oleic, linolic and linolenic acids. Under these HPLC conditions, the separation of the derivatives of myristic and linolic acids is impossible, while the

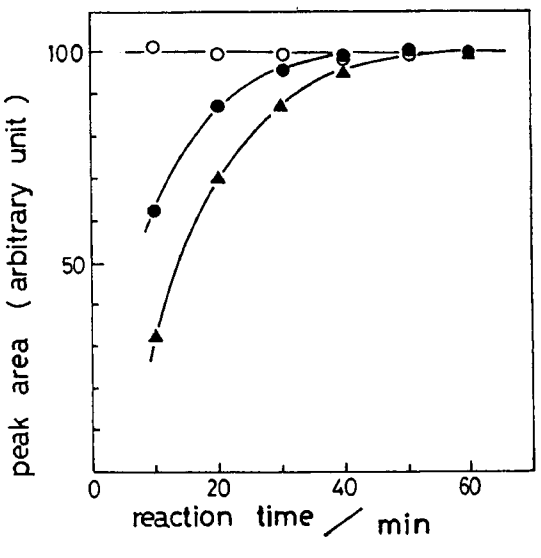

Figure 2 Effect of reaction time on the labelling of capric acid and *n*-octyl mercaptan (○). For labelling of capric acid, labelling reaction was performed in the presence (●) and absence (▲) of 18-crown-6.

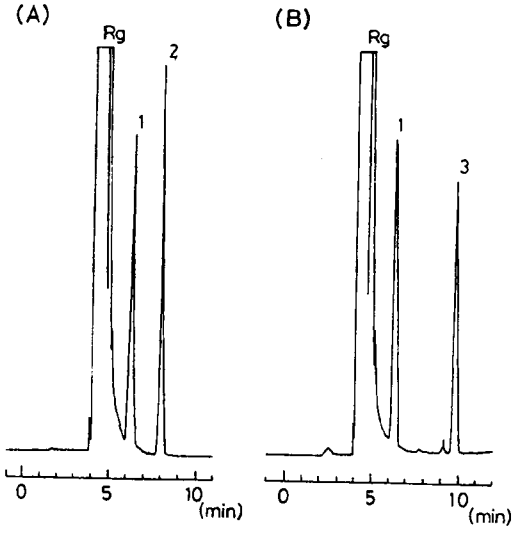

Figure 3 Chromatograms obtained when capric acid (A) and *n*-octyl mercaptan (B) were labelled under the optimum conditions. Peaks: Rg = reagent blank, 1 = 9-phenylanthracene (internal standard), 2 = derivative of capric acid, 3 = derivative of *n*-octyl mercaptan.

derivatives of lauric and linolenic acids can be separated almost entirely. The derivatives of parmitic and oleic acids can be separated incompletely under these conditions. The separation of the derivatives of saturated and unsaturated monocarboxylic acids will be further studied by changing the mobile phase and/or the separation column.

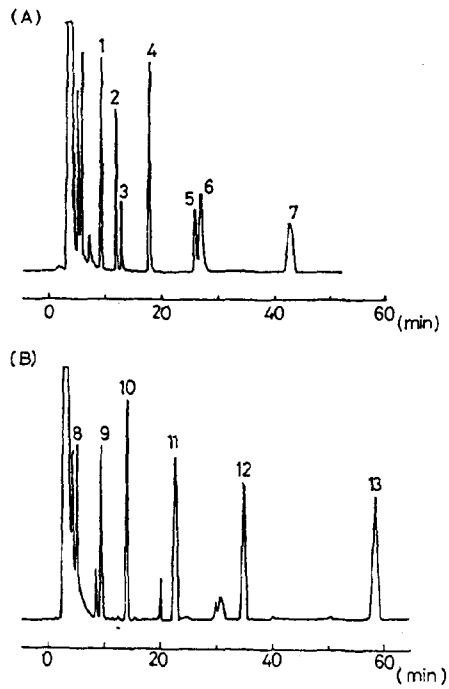

Figure 4 Chromatograms of the mixtures of monocarboxylic acids (A) and mercaptans (B) labelled. Peaks: Rg = reagent blank, 1 = capric acid, 2 = lauric acid, 3 = linolenic acid, 4 = myristic acid + linolic acid, 5 = oleic acid, 6 = parmitic acid, 7 = stearic acid, 8 = *n*-hexyl mercaptan, 9 = *n*-octyl mercaptan, 10 = *n*-decyl mercaptan, 11 = *n*-dodecyl mercaptan, 12 = n-tetradecyl mercaptan, 13 = *n*-hexadecyl mercaptan.

Conclusion

In this work, has been developed a new determination method for monocarboxylic acids and mercaptans which were labelled with 1-(chloromethyl)naphtharene and which were subsequently determined by HPLC. In this work, only aliphatic monocarboxylic acids and mercaptans were treated as samples, but this method would be used for the determination of various compounds containing –COOH and –SH groups. Work is continued. Furthermore, this method is expected to extend to the highly sensitive determination method by using fluorescence detector. The tagging group in this method, 1-naphthylmethyl moiety seems to fluorescence because of the fact that naphtharene fluorescences. Work is also continued.

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