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Methylation of carboxylic acids in supercritical carbon dioxide modified with methanol using a flow-through system

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Abstract

Methylation of carboxylic acids in supercritical carbon dioxide modified with methanol was studied using a flow-through system with and without catalyst. Results without catalyst revealed the reactivity of the fluid itself, although the complete conversion was difficult. Using strong cation-exchanger with H form as catalyst allowed the complete conversion under milder conditions. Parameters, such as temperature, pressure and methanol concentration, were optimized taking account of the rapidity. Applicability of this method combined with solid-phase extraction was demonstrated for the analysis of phenoxyacetic acids in water.

Keywords: Methylation; Supercritical fluid extraction; Carboxylic acids; Phenoxyacetic acids

1. Introduction

Much attention has been focused on the in situ supercritical fluid derivatization and extraction (SFDE). Recent advances were reviewed [1]. Various reactions such as silylation [2], acetylation [3,4], methylation [5–9] are used mainly for subsequent gas chromatographic (GC) analyses. In most cases, derivatization reagents are added onto solid samples in an extraction chamber, if necessary, with catalyst, and supercritical carbon dioxide (CO_2) is used as an extraction fluid. This method reduces the sample preparation time and at the same time enhances the extractability of polar compounds since less polar derivatives are formed and in addition the matrix surface is deactivated. Although extracts can be collected in a small volume of solvent, the volume of the extraction chamber is one of limiting factors for analyzing trace components. One case incorporated a preconcentration step using anion-exchange resin before derivatization of carboxylic acids [8]. The anion-exchange resin, onto which analyte anions in water were concentrated, was placed in the chamber and subjected to SFDE. Such preconcentration can substantially reduces the detection level.

Since solid-phase extraction (SPE) is an excellent method to concentrate trace analytes in water, coupling SPE with supercritical fluid derivatization seems to be attractive. In this case, the on-line method, where analytes on the SPE column are continuously eluted (i.e., extracted) and subjected to derivatization in a flow-through reactor, are more flexible for routine use. One promising fluid which can be used for both elution and reaction is a methanol modified CO_2. It is also one of the most common fluids in supercritical fluid extraction (SFE). Methanol, which is a solvent as well as a reagent for methylation of carboxylic acids in the

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Table 1
Carboxylic acids used for derivatization

Sample[a]	Compound	Abbreviation[b]
I	Benzoic acid	BA
	p-Methoxybenzoic acid	p-OMe
	p-Chlorobenzoic acid	p-Cl
	p-Nitrobenzoic acid	p-NO2
	Lauric acid	C12
	Myristic acid ethyl ester[c]	C14Et
II	Phenoxyacetic acid	PA
	4-Chloro-2-methyphenoxyacetic acid	CMPA
	2,4-Diclorophenoxyacetic acid	2,4-D
	2,4,5-Trichlorophenoxyacetic acid	2,4,5-T

[a] Sample solutions were prepared at 1% in dichloromethane for I and methanol for II.
[b] Abbreviations used in text.
[c] Myristic acid ethyl ester was included for comparison study.

traditional solvent method, was used in SFDE with catalyst such as BF_3 [5], and acidic alumina [9]. Without catalyst, no reactions occurred [6]. This may be due to too low temperature, because recent paper indicated that acid moieties of polyester polymers can be quantitatively converted into their methyl esters in supercritical methanol [10].

In this report, derivatization of carboxylic acids under the continuous flow of supercritical CO_2 modified with methanol are studied with and without catalyst. Using cation-exchanger with H form as a catalyst allowed the complete conversion of various carboxylic acids. Example of on-line SPE and derivatization is also demonstrated.

2. Experimental

2.1. Chemicals

CO_2 was food-additive grade (99.99% purity, Showa Tansan, Yokkaichi, Japan). Methanol and dichloromethane were reagent grade (Kishida, Osaka, Japan). Other chemicals were from Tokyo Kasei (Tokyo, Japan). Carboxylic acids used for methylation are listed in Table 1 together with their abbreviations used in the text. Myristic acid ethyl ester was used for comparison study of transesterification. Corresponding methyl esters were used for GC calibration. Dodecylbenzene was used as an internal standard for GC analysis.

2.2. Delivery of CO_2 modified with methanol

A schematic diagram of supercritical fluid derivatization and extraction system is shown in Fig. 1. A 880-PU pump of Super-200 SFE/SFC system (Jasco, Tokyo, Japan) was used to deliver CO_2. The pump was operated at constant flow mode and the pressure was controlled with a 880-81 back pressure regulator. A split tee was placed before the back pressure regulator and the split flow was directed to the modifier addition unit followed by the sample injection valve. The vented flow from the back pressure regulator was recycled through another tee which was placed before the pump inlet. This operation allowed to decrease the pump pulsation. The modifier addition unit was constructed according to the method [11], which allows one to prepare

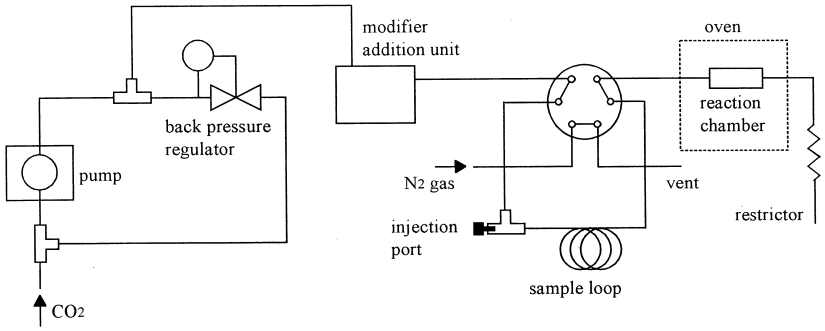

Fig. 1. Schematic diagram of supercritical fluid derivatization and extraction system.

modified CO_2 at constant pressure mode. Modifier was methanol and the concentration was varied in the range of 2 to 20 mol%. The pressure was varied in the range of 60 to 140 atm (1 atm=101 325 Pa).

2.3. Sample introduction

A Rheodyne 6000 valve (Cotati, CA, USA) was used for sample introduction. When introducing samples, sample solvent was removed to avoid affecting the derivatization reaction. For this purpose, a tee was inserted into a midway of the loop attached to the valve. The sample solution (typically 50 μl) was slowly injected through the tee with a micro-syringe while purging with nitrogen gas (400 ml/min). The loop volume (between the tee and the valve) was 100 μl (14 cm×1 mm I.D.). Then, the injection port of the tee was plugged and the deposited solutes were transferred to the reaction chamber by dissolving with modified CO_2. A SPE column (5 cm×4.6 mm I.D.) packed with Varian Bond Elut C_{18} (Harbor, CA, USA) was also used for sample introduction. Water sample was passed through the SPE column at 0.5 ml/min with a MF-2 microfeder (Azumadenki, Tokyo, Japan). The SPE column was purged with nitrogen gas and substituted for the loop.

2.4. Reaction and analytes collection

Two reaction chambers were used. One is a stainless steel tubing (2 m×1 mm I.D.). The other is a stainless steel column (5 cm×4.6 mm I.D.) packed with Varian Bond Elut SCX (silica-based strong cation-exchanger). Before packing, the packing material was washed with 0.1 *M* HCl, water and methanol, and then dried in ambient atmosphere. The reaction temperature was varied in the range of 60 to 200°C.

Effluent from the reaction chamber was bubbled in a 10-ml vial with 5 ml dichloromethane through a fused-silica restrictor (30 cm×25 μm I.D.). Typical flow-rate was about 100 ml/min (as gas-volume) at 100 atm when unmodified CO_2 was used. The resultant solution was evaporated under nitrogen stream, because injecting methanol-containing solution into GC injector lead to methylation of carboxylic acids. The residue was redissolved in dichloromethane (2 ml) and dodecylbenzene was added as an internal standard for GC analysis.

2.5. Gas chromatography

A Hewlett-Packard 5890 series II gas chromatograph with a flame ionization detector was used for determining the conversions of acids and myristic acid ethyl ester to their corresponding methyl esters using splitless injection. Injection volume was 1 μl. The column was an UA-CW capillary column (PEG-20M, 10 m×0.25 mm I.D., 0.25 μm film thickness) obtained from Frontier Lab. (Kohriyama, Japan). The carrier gas was helium. Injector and detector temperatures were held at 300°C. Column pressure was 0.4 atm. Column temperature was held at 60°C for 2 min, and then programmed at a rate of 15°C/min to 250°C. Data analysis was performed with a Shimadzu CR4A integrator (Kyoto, Japan).

3. Results and discussion

3.1. Methylation without catalyst

To examine the reactivity of the supercritical fluid itself, methylation was performed for sample I in Table 1 without catalyst. C14Et was included to examine the transesterification. The results for p-NO2 at 6 mol% methanol are plotted in Fig. 2, which has the highest conversion among benzoic acids. The conversion was increased with increasing temperature and decreasing pressure. Similar trends were observed for other acids, although the extent of conversion was highly dependent on the compound type. The conversion for C12 was comparable to that for p-NO2. The order of decreasing conversion was p-NO2≈C12>C14Et> other acids. Effects of methanol concentration were not significant compared with temperature and pressure.

To interpret the results in Fig. 2, the reaction time should be considered, which corresponds to the time required for sample to pass through the reaction chamber. At constant pressure (i.e., constant mass flow-rate), the reaction time is decreased with raising temperature, because the density is decreased and then the linear velocity is increased. Nevertheless, the conversion was increased with raising tempera-

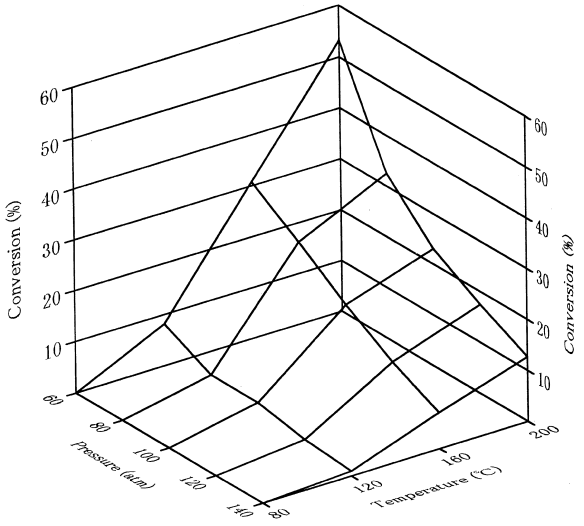

Fig. 2. Effect of temperature and pressure on the conversion in methylation of *p*-nitrobenzoic acid without catalyst. Conditions: 6 mol% methanol in CO_2.

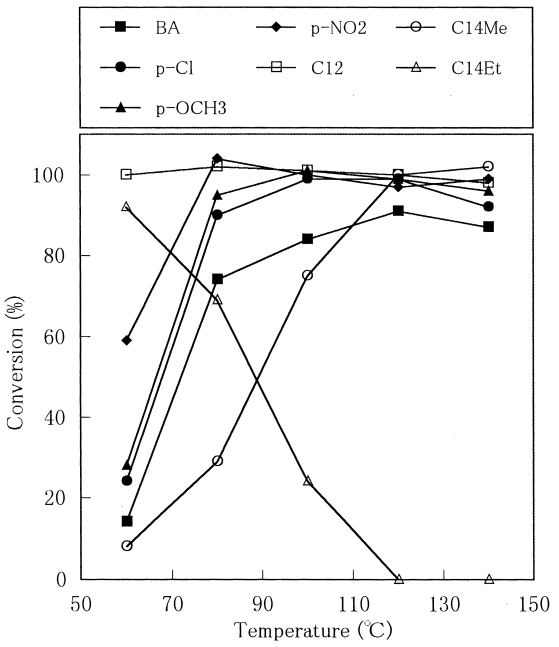

Fig. 3. Effect of temperature on the conversion in methylation of carboxylic acids. Conditions: 140 atm, 10 mol% methanol in CO_2. Cation exchanger with H form was used as a catalyst. C14Me was formed from C14Et by transesterification.

ture This means that raising temperature is very effective. At constant temperature, lowering pressure decreases both the mass flow-rate and the density, which adversely affect the linear velocity. In this condition, the linear velocity was roughly same except for 80°C. Therefore, the pressure effects on the conversion may be due to changes in diffusivity of the fluid.

Although the conversion may be improved by increasing the reaction time, it is unlikely to reach the quantitative level. Adding strong acids (e.g., HCl) as a catalyst will accelerate the reaction, but they must be removed prior to GC analysis. Therefore, strong cation-exchanger with H form was examined.

3.2. Methylation with catalyst

Using cation-exchanger with H form as a catalyst, methylation was performed for sample I in Table 1. The effects of temperature, pressure and methanol concentration on the conversion were examined under various conditions. Fig. 3 shows the effect of temperature at 140 atm and 10 mol% methanol, where unreacted C14Et is also included. Compared with the case without catalyst, the conversion was greatly improved. Even at 60°C, C12 was methylated quantitatively. p-NO2 gave the highest conversion among benzoic acids as in the case without catalyst. Other acids were completely converted at higher than 100°C. Relatively low values for BA are due to the loss on removing the solvent before GC analysis as described in Section 2.4: methyl ester of BA is more volatile than others. This procedure can be eliminated in practical application. Therefore, the relative standard deviation ($n=3$) was relatively large for BA (about 10%), but less than 5% for others. It was confirmed by capillary supercritical fluid chromatography whether unreacted acids were collected. Apart from catalytic effects, one important cause for the improved conversion may be the increased reaction time. Hold-up time at the present conditions was about 5 min. However, much longer time (30–60 min) was required to elute all the solutes. This can be attributed to the strong retention of free carboxylic acids on cation-exchanger. C14Et has the lowest conversion at lower temperature, which is contrary to the results in the case without catalyst. This can be attributed to the less retention of ethyl ester.

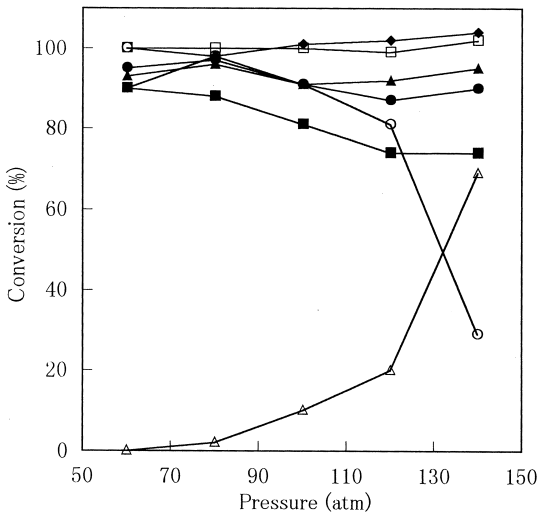

Fig. 4. Effect of pressure on the conversion in methylation of carboxylic acids. Conditions: 80°C, 10 mol% methanol in CO_2. Other conditions and keys as in Fig. 3.

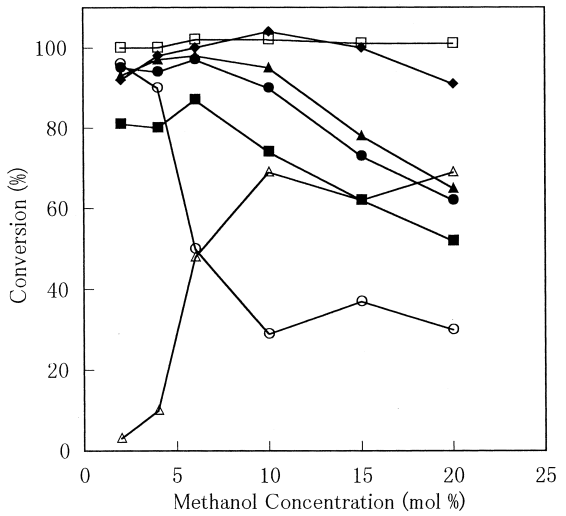

Fig. 5. Effect of methanol concentration on the conversion in methylation of carboxylic acids. Conditions: 140 atm, 80°C. Other conditions and keys as in Fig. 3.

Fig. 4 shows the effect of pressure on the conversion at 80°C and 10 mol% methanol. Although the effect for benzoic acids was ambiguous due to the loss on removing the solvent, the conversion of C14Et was largely increased with decreasing pressure. This can be attributed mainly to the increased reaction time and partly to the increased diffusivity. At 120°C, all the solutes were completely converted in this pressure range.

Fig. 5 shows the effect of methanol concentration on the conversion at 140 atm and 80°C. At less than 6 mol% methanol, all the acids were completely converted. Increase in methanol concentration decreased the conversion. This is due to the decrease in the reaction time rather than the reactivity of the fluid, because such a markedly change was not observed in the case without catalyst. Elevating temperature to 120°C allowed the complete conversion for all the solutes including C14Et in this concentration range.

These results indicate that all the solutes can be completely converted in a wide range of conditions. However, the collection time was highly dependent on the conditions. Considering the rapidity, higher pressure and higher methanol concentration were favorable. Correspondingly, higher temperature was required. Fig. 6 shows time profiles for recoveries of the solutes at 120°C, 140 atm and 20 mol% methanol. Since all the solutes were completely converted under the conditions, relative values were plotted against the time. In this condition, 40 min was required to elute all the solutes, while 150 min was required at 6 mol% methanol.

Applicability of the method in conjunction with SPE was examined for phenoxyacetic acids (sample II in Table 1). The solutes on the SPE column were

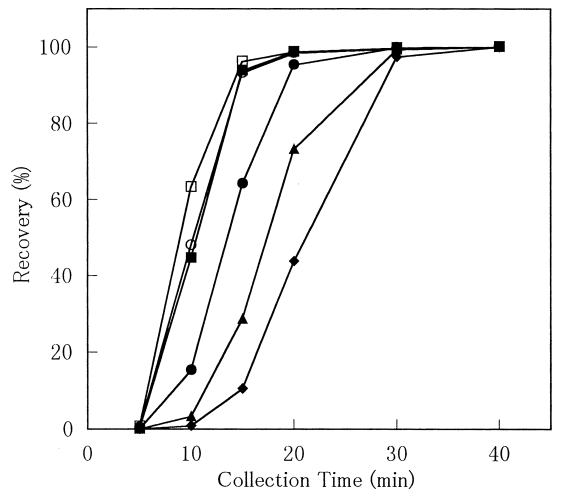

Fig. 6. Plots of recovery vs. collection time in methylation of carboxylic acids. Conditions: 120°C, 140 atm, 20 mol% methanol in CO_2. Other conditions and keys as in Fig. 3.

eluted at 140 atm, room temperature and 20 mol% methanol, and subsequently derivatized at 120°C. Spiking experiments of methanol solution onto the SPE column exhibited the complete conversion within 40 min. When the SPE column was used for concentrating the solutes in water, it was purged and dried at 60°C under nitrogen gas stream (100 ml/min) for 10 min after passing water sample. Water sample was acidified to pH 2 to increase breakthrough volumes. Fig. 7 shows capillary gas chromatograms for a 50-ml water sample spiked with 10 ppb of each and for standards of comparative amounts. The resultant solution after derivatization was concentrated to about 0.2 ml. Although recoveries under the present conditions were not quantitative (less than 50%), this preliminary result indicates that the present method is applicable to trace components in water. Further study on SPE conditions is being performed.

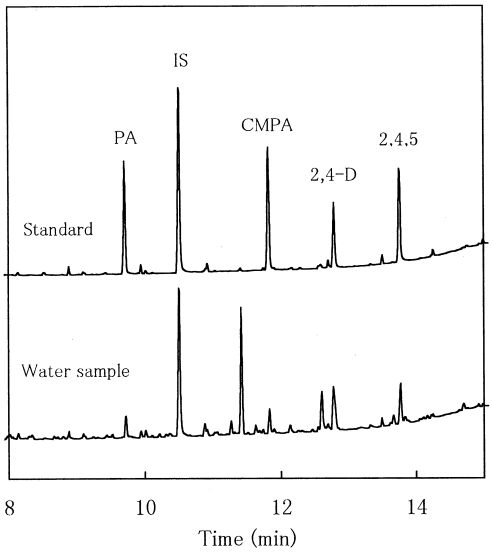

Fig. 7. Capillary gas chromatograms of phenoxyacetic acid methylesters. Acids were extracted from water (50 ml, pH 2) spiked with 10 ppb each using SPE and derivatized with the present system. Conditions: 120°C, 140 atm, 20 mol% methanol in CO_2. Samples: phenoxyacetic acid (PA), 4-chloro-2-methyphenoxyacetic acid (CMPA), 2,4-diclorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

4. Conclusions

Carboxylic acids can be methylated quantitatively in supercritical CO_2 modified with methanol using strong cation-exchanger with H form as a catalyst. The resultant solution dose not contain interference materials for GC analysis, making the following analysis simple. The method combined with SPE can be applied to the analysis of trace components in water.

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