On-Line Coupling of Gas Chromatography-Gas Chromatography for the Determination of Coumarin in Kerosene

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Abstract
Determination of coumarin in kerosene was studied. Kerosene is discriminated from taxed fuel oil by the addition of coumarin in Japan. A fluorescence detection method has been widely used to check the legal distribution of kerosene, however, the method is inevitably resulting waste fluid consisted both of the aqueous alkaline solution and the organic solvent. By introducing a heart-cutting analysis with a set of on-line coupled packed-capillary and open-tubular columns, coumarin in kerosene was successfully determined in gas chromatography. The developed technique showed several advantageous features such as a significantly reduced waste, quick and easy separation/determination, along with a reduced sample volume required in the determination process.

Keywords: Illegal diesel fuel; Coumarin; Heart-cutting; Gas chromatography; Fluorescence; Mass spectroscopic detector

1. Introduction
Diesel fuel used for automobiles is subject to a local tax called "light oil tax" in Japan and the price of the diesel fuel sold at gas stations includes this local tax. However, there are illegal businesses attempting to avoid the mandatory tax payment by mixing non-taxable kerosene to a diesel fuel. For a legal and orderly distribution of the diesel fuel, coumarin has been added to kerosene as a recognition marker in Japan since 1991. The coumarin in kerosene is normally detected under the official determination protocol prescribed by National Petroleum Association in Japan [1-3].

It has been pointed out that the difficulty in the waste treatment is one of the disadvantages of the above official fluorescence method, because the method requires a mixture of an organic solvent and an aqueous alkaline solution. In addition, the official method could not be used if the sample volume is limited. The official method has been modified to be a brief procedure with the organic solvent, but without the extraction process, in the field of forensic chemistry [2]. The detection of coumarin was carried out by adding only an alkaline solution to the sample, although the measurement of the fluorescence spectra of coumarin derivatives is somewhat difficult due to the interferences with other fluorescent components in oil samples [4].

High-performance liquid chromatography (HPLC) has also been used for the analysis of coumarin [5], however, this method has some the disadvantages, such as a time-consuming sample preparation and the requirement of a large amount of organic solvent. Another technique to detect illegal diesel fuel was employed, where the sulfur content of the petroleum sample was determined using an X-ray fluorescence instrument or an atomic emission detector. The method is only applicable to diesel fuel that has been produced by a de-coumarin treatment in advance [6]. Therefore, coumarin must be eliminated before the analysis.
Gas chromatography (GC) has been widely employed for the separation and determination of various volatile compounds. GC has widely been employed in various fields of science, and many types of stationary phases have been developed and commercialized in the past several decades along with a numerous number of applications [7,8]. In recent GC separations, open-tubular capillary columns were mainly employed for the precise separation of complex sample mixtures due to its high separation performance, although conventional packed-columns are still used for the separation of a certain class of compounds, especially for highly-volatile compounds on the basis of the unique retentivity and selectivity [9].

In packed-column GC, a wide variety of stationary phases can be employed [7-25] including fine polymeric fibers having a good heat-resistance [13-25], and therefore, various types of volatile compounds having different polarities as well as permanent gases were successfully separated with a reasonable analysis time [10-25]. The sample loading capacity of the packed-column is also quite remarkable. In contrast to the above-mentioned advantageous features of the packed-column in GC, the attainable maximum theoretical plate number of typical packed-column is somewhat limited. In addition, the temperature-programmed separation of a conventional packed-column is also limited because of the relatively large heat capacity of the packed-columns.

To obtain a complete separation of a complex sample mixture, column switching or multidimensional gas chromatographic (MDGC) analysis has been widely introduced [26,27]. In most of the recent MDGC, an open-tubular capillary column has been employed to accomplish a better separation of the complex sample mixture. MDGC analysis using two or more capillary columns provides a significantly greater separation power, along with a good compatibility to a conventional mass spectrometric detector (MSD). Column switching analysis using conventional packed-columns is still carried out for difficult separation problems that are not solved by a simple GC separation on single column, such as the determination of impurities [28-30].

In our previous investigations, novel packed-capillary columns for a rapid temperature-programmed GC analysis were developed with a thin-wall stainless-steel capillary of 1.0 mm i.d. [10-12]. The developed packed-capillary columns can be installed to a conventional capillary GC system without any modifications and adapters. These columns showed a unique selectivity on the basis of the packing material, along with a good sample loading capacity. Applications of the packed-capillary column includes the evaluation of photocatalytic activity of TiO2 [31], and heart-cutting separation of complex sample mixtures [12]. In this work, a novel GC determination technique of coumarin in commercially-available kerosene samples was investigated. With a heart-cutting analysis using a set of on-line coupled packed-capillary and open-tubular columns, coumarin in kerosene was determined in GC-GC-MSD.

2. Experimental
2.1. Reagents and solvents
All of the reagents and organic solvents were of analytical reagent grade, and were obtained from Tokyo Chemical Industry (Tokyo, Japan). The kerosene and light diesel oil samples were purchased from several local gas stations as described below. For the reference, a kerosene standard solution was prepared by adding 1.0 mg/L (1.0 ppm) of coumarin to an alkane mixture, where the carbon numbers of these alkanes are from 11 to 15. Water was purified by a Milli-Q Water Purification System (Millipore, Darmstadt, Germany).

2.2. Fluorescence analysis
For the fluorescence analysis of coumarin in the fuel [1-3], a mixture of alcohol containing ethanol and butanol with a volume ratio of 4:3 was prepared, and then the resulting alcohol mixture was mixed with an aqueous alkaline solution separately prepared, where the aqueous alkaline solution was prepared by dissolving 10 g of sodium hydroxide and 20 g of sodium nitrate in 100 mL of water. Fifteen mL of fuel sample was mixed with the above solvent mixture consisted of 15 mL each of the alcohol mixture and the aqueous alkaline solution. Coumarin existed in the fuel was hydrolyzed with the aqueous alkaline solution to yield cis- or trans-o-hydroxycinnamate anion. Then, the resulting cis- or trans-o-hydroxycinnamate anion remained in the aqueous phase, while most of the oil components were extracted with the organic phase consisted of ethanol and butanol. By irradiating UV light of 365 nm to the organic phase, trans-o-hydroxycinnamate anion is formed by the isomerization, allowing a visual observation of yellow-green fluorescence (ca. 500 nm) emitted from the compound as illustrated in Fig. 1.

![Fig. 1. Reaction scheme of coumarin to cis- or trans-o-hydroxycinnamate anion.](image-url)
2.3. Packed-capillary column

Packed-capillary columns were prepared with a conventional manner as described previously [10-12] except for the use of a stainless capillary of 1.0 mm i.d., 1.27 mm o.d., 1.0 m length and a packing material having a relatively smaller particle diameter as described below, where with a careful packing, the resulting column efficiency per a unit length was almost the same as that of typical conventional particle-packed columns. A pair of stainless steel capillaries of 0.3 mm i.d., 0.52 mm o.d., 0.5 m length were attached to the inlet and outlet of the packed-capillary column, allowing an easy installation of the packed-capillary column to a conventional GC instrument designed for capillary column connection.

For the preparation of packing material, a Shimalite W, white diatomaceous earth, acid washed and subsequent dimethylchlorosilane (DMCS) treated (Shinwa Chemical Industries, Kyoto, Japan), was used as the support. The spherical porous particles between 100 and 80 mesh, corresponding to about 150 and 180 µm in diameter, respectively, were sieved and the specific surface area of the material was about 0.70 m²/g. Among various types of liquid phases for packed column, one of the following liquid phase was employed on the basis of the results in preliminary experiments: Silicone SE-30, Silicone SE-52 or Polyethylene glycol PEG-20M (Shinwa Chemical Industries). For the coating onto the support material, a hexane solution of liquid phase was used, where the weight of the liquid phase was set at 5%-weight of the support material. The resulting packing materials were packed into the stainless-steel capillary as described above. These columns were employed with an appropriate preconditioning at 300ºC for 60 h before use, and no significant bleeding were observed in the temperature range up to 300ºC after this preconditioning procedure.

2.4. Heart-cutting GC system

Two Agilent 6890N gas chromatographs (Agilent Technologies, Mountain View, CA, USA) with a flame ionization detector (FID) were used for the GC measurements. The heart-cutting system was developed with two columns connected by a commercially available 6-way valve (GL Sciences, Tokyo, Japan) as illustrated in Fig. 2. In order to avoid any undesirable adsorption inside of the valve, a 6-port valve was placed in a GC oven, where the valve position could be manually switched from the outside of the GC oven. The connection between columns and valve was established using conventional stainless-steel capillary of 0.3 mm i.d., 0.52 mm o.d., 0.5 m length.

To make sure the complete separation of all analytes, the valve operation was optimized as follows. When the target fraction was just passing through the valve, the position of the valve was changed to transfer the fraction to the 2nd column, and the valve position was then returned to the original position to ensure the separation of other fraction in the 1st column. With the above operation, these two fractions could be detected in the corresponding GC detector placed at the end of each column. The relative standard deviations (RSDs) of the retention time were less than 2.0% (n = 5) in all of the columns when operating either switching or heart cutting analysis.

As the carrier gas, either N₂ or He was used, where N₂ or He and air were supplied from the respective gas cylinders through a cartridge packed with molecular sieve (Kishida Chemical, Osaka, Japan). All of the injections were operated in a split mode with a ratio of 5:1. The injector and detector were set at 280ºC. In order to maintain the system during the sample loading step, a section of metal capillary column was used as the resistance tube as shown in Fig. 2. The samples were injected in amounts of 1.0 µL. The other separation conditions such as carrier gas flowrate, column head pressure, and temperature programs were determined by the results of preliminary experiments for each sample. The data collection was made with Borwin data handling software (Jasco, Tokyo, Japan) running on a personal computer.

2.5. MSD analysis

For heart-cutting GC-GC-MSD measurements, the above system was hyphenated to an HP 5973 MSD (Agilent Technologies). As the transfer line, a stainless-steel capillary of 0.3 mm i.d., 0.52 mm o.d., 0.8 m length was used, where inlet of the transfer line was connected to the
end of the second column and the other end was connected to the MSD. The system overview is illustrated in Fig. 3.

GC-MSD interface temperature and the ionization voltage were set at 250°C and 70 eV, respectively, and a typical electron impact ionization was employed. The MSD was operated in a selected ion monitoring mode set at m/zs of 90, 118 and 146. Scan time was controlled from 38 min to 50 min. The data collection was made with Chemstation data handling software (Agilent Technologies).

3. Results and discussion

3.1. Detection of coumarin by conventional fluorescence analysis

First, the existence of coumarin in commercially available fuels was confirmed by a conventional fluorescence analysis. The results of the fluorescence analysis of coumarin in each fuel sample using the conventional method are shown in Fig. 4. It was confirmed that all the samples shown here were separated from the upper oil layer, the middle alcohol layer and the lower alkaline solution layer. In this procedure, trans-o-hydroxycinnamate anion is extracted to the alkaline solution layer. A characteristic yellow-green fluorescence emitted from the coumarin in the lower layer was observed for the standard solution and kerosene, showing the existence of coumarin. In addition, no fluorescence was observed in the alkaline solution layer of a light diesel fuel without adding coumarin therein. Therefore, as expected, it was confirmed that the above fluorescence procedure could be used to confirm whenever coumarin is present or not.

However, the determination of coumarin in the above method is somewhat difficult due to a complicated manual multistep sample preparation procedure. In order to avoid a complex waste treatment, it is necessary to develop an alternative analytical procedure because the conventional technique still needs the preparation of a mixture of organic solvent and aqueous alkaline solution.

3.2. Determination of valve switching time

Fundamental performance of the instrument was confirmed in the previous work for the system equipped with two FIDs (Fig. 2) [18], where a standard sample mixture is consisted of ca. 9% each of 11 organic solvents commonly found in automobile fuels was separated with a heart-cutting operation on SE-30 and PEG-20M packed-capillary columns. It was confirmed that the desired fraction was transferred to the second column [18].

To prevent the flow of typical kerosene component into the MSD, valve switching time was optimized on a similar system equipped with two FIDs as described above. First, standard sample and kerosene containing 100 mg/L of coumarin were analyzed on first column (SE-52 packed capillary) only. Several test runs were good enough to find the optimized switching time, as long as the elution time from the first column was successfully obtained. As the result, valve switching time was set at 1530 s for changing the position as illustrated in Fig. 2A to that in Fig. 2B and then returned to the original position at 1800 s. The heart-cutting analysis is successfully carried out for kerosene samples and the prepared kerosene samples spiked with 100 mg/L of coumarin in the conditions.

Fig. 3. On-line coupled heart-cutting GC-GC separation system having an MSD with a specially-designed transfer line from GC. At the interface between GC and MSD, a home-made ribbon heater was used. Valve position: (A) separation on the first column or that on two individual columns, (B) sample loading for the separation on the second column.

Fig. 4. Analysis of coumarin in fuel samples using the conventional fluorescence method. All the kerosene samples from (A) to (F) were obtained from local gas stations as summarized in Table 1. For reference a light diesel oil was also obtained from the same gas station as (C). Standard solution was prepared as an alkane (C11-C15) mixture containing 1.0 mg/L as described in the text.
Typical chromatogram for the separation of this coumarin-spiked kerosene sample is shown in Fig. 5. Fig. 5B shows a successful transfer from first column to second column (DB-WAX of 15 m × 0.25 mm i.d., 0.25 μm, J and W Scientific) and also subsequent good separation of fraction including coumarin. These results suggest a successful separation of typical kerosene sample. Similar experiments for other kerosene samples were carried out, and a good separation of typical kerosene components was confirmed along with the confirmation of coumarin in all the kerosene samples studied.

3.3. Heating of transfer line

A heart-cutting analysis of the standard solution was carried out, however, the resulting data showed that the sensitivity has quite lower than a typical MSD sensitivity. It is considered that the cause of lower sensitivity is a low temperature in the transfer line. In order to make sure an uniform heating and to maintain a high temperature, instead of the conventional tube-shaped heater equipped in the original GC-MSD interface, a ribbon heater was introduced for the heating of transfer line in the following experiments. The ribbon heater was directly wrapped around a transfer line and temperature was set at 250ºC.

3.4. Digital noise filter

Because of a relatively lower concentration, the detectability of coumarin should be improved to ensure the successful determination of real kerosene samples. In this work a digital noise filter running on a personal computer
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was introduced. As a noise filter, a digital low pass filter was introduced, where high frequency elements more than constant frequency were removed. However, too low cut-off frequency induced a poor peak shape, while too high cut-off frequency increased the noise. Therefore, the cut-off frequency was optimized on the basis of the systematic monitoring the peak area of coumarin during the cut-off frequency was changed as illustrated in Fig. 6.

The resulting plot shows that the peak area will be saturated at a certain cut-off frequency level. On the other hand, too high cut-off frequency introduced an increase in the noise level. Therefore, the cut-off frequency was optimized as 0.136 Hz. As compared to the chromatogram before the filtering, noise is reduced with the peak shape of coumarin in the chromatogram maintained. Therefore, it can be concluded that the above selective noise removal was successfully done without a significant deterioration of the chromatogram as typically shown in Fig. 7.

The concentration of coumarin in kerosene sample was determined using the above GC-GC-MSD system equipped the digital noise filter in the data processing system, as summarized in Table 1, where two kerosene samples (A and B) were obtained from different gas station but the same oil supplier. About 1.0 mg/L (1.0 ppm) of coumarin was successfully determined for all the kerosene samples studied.

The lowest limit of detection (LOD) and the lowest limit of quantification (LOQ) are 0.11 mg/L and 0.36 mg/L, respectively. The results clearly demonstrated that the heart-cutting analysis will be a promising alternative technique for the determination of coumarin in kerosene, although a further sensitivity enhancement might be needed.

![Fig. 7. Typical separation of kerosene sample A on the heart-cutting separation in the second column (DB-WAX). Detection was made with MSD, and the subsequent data analysis was carried out with the digital noise filter running on the PC. Carrier gas: He. Temperature program in the 1st dimension: 40ºC (2 min) to 230ºC (10 min) at 5ºC/min. Temperature program in the 2nd dimension: 40ºC (32 min) to 160ºC at 40ºC/min and then 160ºC to 230ºC (8 min) at 10ºC/min. Valve switching time: 1530 s (corresponding the valve position from Figs. 3A to 3B) and 1800 s (corresponding the valve position from Figs. 3B to 3A). Other conditions are described in the text.](image)

<table>
<thead>
<tr>
<th>sample</th>
<th>location of gas station</th>
<th>supplier</th>
<th>coumarin concentration (mg/L)</th>
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<tr>
<td>A</td>
<td>Tatsuno, Nagano</td>
<td>I</td>
<td>0.92</td>
</tr>
<tr>
<td>B</td>
<td>Nagoya, Aichi</td>
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<td>0.87</td>
</tr>
<tr>
<td>C</td>
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<tr>
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<td>V</td>
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</table>

4. Conclusions

In this work, successful separations of coumarin in kerosene samples were accomplished by the column switching analysis in conventional capillary GC systems with a set of on-line coupled packed-capillary and open-tubular columns. Introducing a MSD, the determination of coumarin is also demonstrated for commercially available kerosene samples.

The developed technique has various advantages over the conventional fluorescence analysis: i.e. alkaline solution, organic solvent and sample preparation process are not required, while the determination of coumarin in a small amount of sample could be done. Further applications of the heart-cutting analysis with the packed-capillary columns, including the separation of other taxed diesel such as A-type heavy oil could be expected with a further modification in the heating system in the transfer line along with the optimization of the separation conditions such as the use of a high separation temperature.

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