

## Application and Validation of a Determination Method Using Post-column Reaction Gas Chromatography of Nitrogen-containing Organic Compounds

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In this work, we applied post-column reaction gas chromatography (GC) using a flame ionization detector (FID) system to study nitrogen-containing organic compounds (NOCs). The results were subsequently validated. After separation by column, the target components were converted to carbon dioxide using an oxidizing catalyst and then reduced to methane, followed by detection using an FID. SI-traceable testing mixtures containing NOCs (isopropcarb, napropamide, and pendimethalin) were prepared by the gravimetric blending method. These mixtures were analyzed using a post-column reaction GC-FID system; standard materials of hydrocarbons were used as calibrants in this analysis. The determined values were compared with the values obtained for samples prepared at the corresponding concentrations, and statistical analyses were performed in all cases. It was shown that the determined and prepared values agreed well with each other within the uncertainty limits.

**Keywords** Post-column reaction gas chromatography, flame ionization detector, GC-FID, nitrogen-containing organic compound, validation, SI-traceable

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### Introduction

Nitrogen-containing organic compounds (NOCs) are widely utilized in our daily life as dyes, drugs, fertilizers, alkaloids, proteins, *etc.* In general, NOCs are more chemically active than saturated hydrocarbons. As many NOCs are harmful to the human body, their carcinogenic risk has been scientifically assessed and summarized by independent working groups.<sup>1</sup> From the viewpoint of occupational safety and health, exposure limits for chemicals including NOCs have been released by various occupational health organizations.<sup>2-4</sup> Furthermore, the manufacture and use of NOCs, such as benzidine and 2-naphthylamine, have been prohibited in several countries due to their strong carcinogenicity.<sup>1</sup> However, when controlling the quality of products, such as dyes, analysts sometimes need to directly handle hazardous NOCs to calibrate their analytical instruments<sup>5-10</sup> because the sensitivity of commonly used systems, such as a gas chromatography (GC)-flame ionization detectors (FIDs), differs for each target component. Therefore, the development of safer analytical methods that do not require analysts to directly handle hazardous NOCs is required.

We have developed a post-column reaction GC-FID system, which is composed of a GC-FID apparatus as well as oxidizing and reducing parts, located in series.<sup>11-14</sup> Applications of post-column reaction GC-FID systems have also been reported by

other research groups.<sup>15-19</sup> In this system, target components separated by column are completely converted to carbon dioxide at the oxidizing part and then completely reduced to methane, followed by detection using the FID. Therefore, the sensitivity is proportional to the number of carbon atoms of a target component, irrespective of the compound, and it is not necessary to prepare all the standard materials related to the target components to calibrate the system. Quantitative analysis of hazardous and difficult-to-handle components can be performed using this system; this analysis is calibrated with low-toxic and easy-to-handle standard materials. Although validation of the determination method using the post-column reaction GC-FID system was reported, the target components were limited to volatile organic carbons, which consist of three elements, namely, carbon, hydrogen, and oxygen.<sup>13,14</sup> Applications for measuring NOCs have been reported before.<sup>17-19</sup> However, performances of the determined values were not sufficient in accuracy and precision. The reported result had a too large error (over 8% relative)<sup>17</sup> compared with the hydrocarbons measurement results (relative standard uncertainties of measured values: 0.15–2.10%),<sup>15</sup> and the obtained value was not proportional to the number of carbon atoms in the target component (the effective carbon number was  $0.96 \pm 0.01$ , not 1.00),<sup>18</sup> therefore it is presumed that there was some bias or fault in the results. Only the average measurement error (2.6%) and the estimated standard deviation of measurement error (0.91%) were shown, and concrete measurement errors of each target species were not indicated.<sup>19</sup> It remained obscure whether a post-column reaction GC-FID system was applicable for

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measuring NOCs. In this work, we applied post-column reaction GC-FID systems to the analysis of NOCs, which consist of four elements, namely, nitrogen, carbon, hydrogen, and oxygen, and validated the results using SI-traceable NOC standard materials. The studied NOCs were all compounds that are volatile; they are also pesticidal and toxic to aquatic life.<sup>20</sup> Target accuracy for this study is that obtained values by the post-column reaction GC-FID systems are proportional to the number of carbon atoms in the target component. Target precision is that obtained relative uncertainties of values were similar with the uncertainties of measured values of hydrocarbons.<sup>15</sup>

## Experimental

### Reagents and chemicals

A certified reference material, *o*-xylene [NMIJ CRM 4011-a, National Metrology Institute of Japan (NMIJ)], was used in this work. Traceable reference material grades of isoprocarb (2-isopropylphenyl methylcarbamate; C<sub>11</sub>H<sub>15</sub>NO<sub>2</sub>; a carbamate component), napropamide (*N,N*-diethyl-2-(1-naphthoxy)-propionamide; C<sub>17</sub>H<sub>21</sub>NO<sub>2</sub>; an amide component), and pendimethalin (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzeneamine; C<sub>13</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>; a component including amine and nitro functional groups) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); characteristic values (purities) of these three components were traceable to the SI unit. Tetradecane, hexadecane, and octadecane were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and the purities of these three components were provided from the NMIJ Calibration Service. The purities of the above seven components (together with their uncertainties) are summarized in the Supporting Information (Table S1). Acetone (JIS special grade), purchased from Wako Pure Chemical Industries, Ltd., was used as the solvent.

### Instruments

Two post-column reaction GC-FID systems were used in this work. One of them (system A) was a combination of a GC-FID unit (GC-2010, Shimadzu Corporation, Kyoto, Japan) and handmade oxidizing and reducing parts, and has been reported previously.<sup>15</sup> The other one (system B) was composed of commercial oxidizing parts and reducing parts (TE-1000, HORIBA STEC, Co., Ltd., Kyoto, Japan) and a GC-FID unit (6890A, Agilent Technologies, Santa Clara, CA, USA). An InertCap 5MS/Sil+TL capillary column (GL Sciences Inc., Tokyo, Japan) and a DB-5MS capillary column (Agilent Technologies) were used for the separation. The operating conditions of the post-column reaction GC-FID systems are summarized in the Supporting Information (Table S2). In system B, the carrier gas flow-rate control was set to the "ramped pressure" mode because the flow rate cannot be adjusted by the septum purge regulator of the gas chromatograph (Agilent 6890A).

### Preparation of testing mixtures and intermediate mixtures

The preparation scheme for testing and intermediate mixtures is shown in Fig. 1. All mixtures were prepared using the gravimetric blending method.<sup>21</sup> An AG245 balance (capacity: 41/210 g, resolution: 0.01/0.1 mg; Mettler-Toledo International Inc., Greifensee, Switzerland) was used for the preparation of all mixtures. The preparation procedures are detailed in the Supporting Information. The concentrations of all the components, expressed as methane equivalents, and the

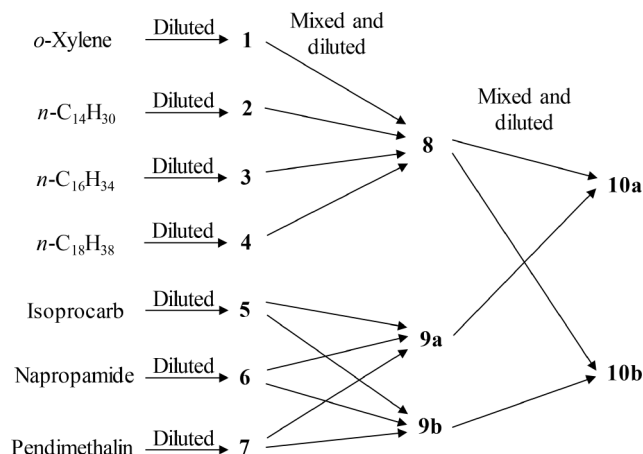


Fig. 1 Preparation scheme of testing mixtures and intermediate mixtures.

Table 1 Concentrations of components in testing mixtures **10a** and **10b** (including uncertainties)

Component	Concentration ( $\mu\text{mol kg}^{-1}$ of CH <sub>4</sub> equivalent) <sup>a,b</sup>	
	<b>10a</b>	<b>10b</b>
<i>o</i> -Xylene	7576 $\pm$ 6 (0.08%)	7406 $\pm$ 6 (0.08%)
Tetradecane	5514 $\pm$ 55 (1.01%)	5391 $\pm$ 54 (1.01%)
Hexadecane	4747 $\pm$ 29 (0.61%)	4641 $\pm$ 28 (0.61%)
Octadecane	6438 $\pm$ 65 (1.01%)	6294 $\pm$ 64 (1.01%)
Isoprocarb	5609 $\pm$ 63 (1.12%)	5776 $\pm$ 65 (1.12%)
Pendimethaline	4770 $\pm$ 30 (0.63%)	4934 $\pm$ 31 (0.63%)
Napropamide	6635 $\pm$ 74 (1.12%)	6696 $\pm$ 75 (1.11%)

a. The numeric value after the symbol  $\pm$  of each mean value indicates an expanded uncertainty ( $k = 2$ ).

b. Figures in parentheses represent relative expanded uncertainties ( $k = 2$ ).

uncertainties for the testing mixtures **10a** and **10b** are summarized in Table 1. Amounts of components in the testing mixtures injected into the post-column reaction GC-FID systems are shown in Table S4 (Supporting Information).

## Results and Discussions

Four hydrocarbons (*o*-xylene, tetradecane, hexadecane, and octadecane) were used as internal calibration standards in the testing mixtures. The concentrations of three NOCs (isoprocarb, pendimethalin, and napropamide) were determined in the samples by an internal-standard method using the post-column reaction GC-FID system.

Initially, the efficiencies of the oxidation reaction in both systems, A and B, were evaluated for all the components in the testing mixtures. Samples **10a** and **10b** were measured by systems A and B, respectively. During the evaluations,

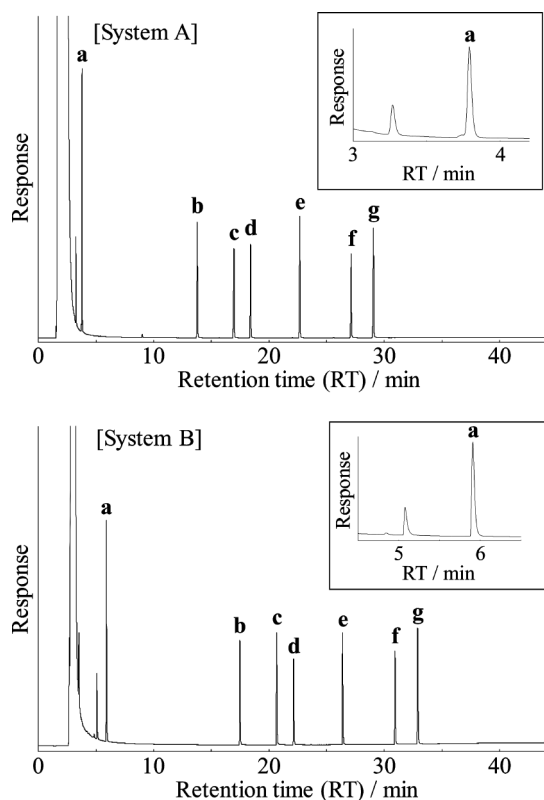


Fig. 2 Chromatograms of the testing mixtures **10a** (by system A) and **10b** (by system B). The chromatograms are shifted and multiplied for clarity: **a**, *o*-xylene; **b**, tetradecane; **c**, isoprocarb; **d**, hexadecane; **e**, octadecane; **f**, pendimethalin; **g**, napropamide.

the second reaction parts in the instruments were bypassed, and then all the components were converted to carbon dioxide and were introduced into the FID. We did not observe any peaks corresponding to the components of the mixture; this indicates that the oxidation reaction occurred perfectly since the FID does not respond to carbon dioxide.

In this study, nitrogen atoms were contained in target components, and degradation of both oxidizing and reducing catalysts by the nitrogen atoms could be considered. Degradation of oxidizing and reducing catalysts in both systems A and B was not found in this study. If nitrogen oxides were generated, the catalysts, especially the reducing catalysts, would be damaged and the reactions would not occur perfectly. It seems that carbon dioxide, water, and nitrogen were generated by the oxidation reaction of the NOCs. In both systems A and B, purified air was used as the oxidizer, and the catalysts in the systems were in contact with nitrogen in the air. Therefore, the generated nitrogen in the oxidation reaction did not influence the catalysts.

The testing mixtures were analyzed using both the oxidation and reduction parts. The obtained chromatograms are shown in Fig. 2. Since a chromatographic peak for *o*-xylene overlapped with the signal from one of the unknown components in system B, we did not use *o*-xylene as an internal calibration standard. Regression lines between the concentrations of the internal calibration standards and the response of the FID were prepared using Deming's least-square method, which is described in ISO 6143.<sup>22</sup> The linearity of the regression lines was validated by measuring the goodness-of-fit ( $\Gamma$ ) with respect to ISO 6143. The parameters of the regression lines and the  $\Gamma$  values are

Table 2 Parameters derived from the regression lines

	Expression $y = a + bx^a$				Goodness-of-fit ( $\Gamma$ )
	$a$	$u(a)$	$b$	$u(b)$	
System A	$-1.325 \times 10^4$	$2.562 \times 10^4$	178.7	4.9	0.11
System B	14.01	15.73	0.0856	0.0030	0.59

a. Unit of  $x$  is  $\mu\text{mol kg}^{-1}$  of  $\text{CH}_4$  equivalent. Units of  $y$  in system A and system B are  $\mu\text{V s}$  and  $\text{pA s}$ , respectively.

Table 3 Comparison of reference values for the concentrations of target components in the testing mixture with the results obtained by post-column reaction GC-FID

Post-column reaction GC-FID system	Testing mixture	Target component	Concentration ( $\mu\text{mol kg}^{-1}$ of $\text{CH}_4$ equivalent) <sup>a,b</sup>		$E_n$
			Reference	Obtained	
System A	<b>10a</b>	Isoprocarb	$5609 \pm 63$ (1.12%)	$5585 \pm 63$ (1.14%)	0.27
System A	<b>10a</b>	Pendimethaline	$4770 \pm 30$ (0.63%)	$4747 \pm 58$ (1.22%)	0.36
System A	<b>10a</b>	Napropamide	$6635 \pm 74$ (1.12%)	$6649 \pm 102$ (1.53%)	0.11
System B	<b>10b</b>	Isoprocarb	$5776 \pm 65$ (1.12%)	$5845 \pm 111$ (1.90%)	0.54
System B	<b>10b</b>	Pendimethaline	$4934 \pm 31$ (0.63%)	$4961 \pm 77$ (1.56%)	0.32
System B	<b>10b</b>	Napropamide	$6696 \pm 75$ (1.11%)	$6803 \pm 159$ (2.34%)	0.61

a. The numeric value after the symbol  $\pm$  of each mean value indicates an expanded uncertainty ( $k = 2$ ).

b. Figures in parentheses represent relative expanded uncertainties ( $k = 2$ ).

summarized in Table 2. Both  $\Gamma$ s in Table 2 were below the critical value of 2, and they also indicated good compatibility of the regression lines with the calibration data. We demonstrated that the post-column reaction systems were functioning correctly. The regression lines were used as calibration lines, and the concentrations of the three NOCs in the testing mixtures were determined. The calibration line was extrapolated for the analysis of pendimethalin. The uncertainties of the determined values include uncertainties coming from the concentrations of the calibrants, the preparation of testing mixtures, the repeatability of the measurements, and the parameters of the calibration lines. The determined values were compared with the prepared values at the corresponding concentrations, and a statistical inspection of the determined values was performed using the  $E_n$  number, which is described in ISO/IEC 17043.<sup>23</sup> The obtained results are summarized in Table 3, and the sensitivity of the systems is in Table S5 (Supporting Information). The main factors affecting the uncertainties for the obtained results are the repeatability of the measurements and the uncertainties of used internal calibration standards such as tetradecane, hexadecane and octadecane in the testing mixtures. The uncertainties obtained in system B were all larger than those obtained in system A. Since the injected amount of the testing mixtures into the system B was smaller than that into the system A, the uncertainties related to the repeatability of the measurements seem to be larger. Because of extrapolation of

the calibration in the analysis of pendimethalin, uncertainties of the measurement of pendimethalin were relatively larger than those of isoprocarb and napropamide. All  $E_n$  values were below one, and it means that the determined and prepared values were consistent with each other within the uncertainty limits. In other words, the determined values by the post-column reaction GC-FID systems were proportional to the number of carbon atoms in the target component, and our determination method has been validated and can be applied to evaluate concentrations of NOCs using hydrocarbons as calibrants.

Our results show that the obtained values were proportional to the number of carbon atoms in the target components and the uncertainties for the obtained results (relative expanded uncertainty: 1.18 – 2.34%, ( $k = 2$ )) included not only the uncertainties for the repeatability of the measurements but also the uncertainties for the concentrations of the internal calibration standards. The obtained relative uncertainties were similar with the uncertainties of measured values of hydrocarbons.<sup>15</sup> It means that our results were more accurate and precise than those reported by other groups.<sup>17–19</sup> There were some possible reasons for demonstration of better performance in this study. Firstly, the efficiencies of the oxidation part in our systems were evaluated and it was confirmed that the oxidizing reaction proceeded completely. On the other hand, in the reactors reported before,<sup>17–19</sup> it is not possible to evaluate the efficiency of the oxidizing part because of a structural reason, and the oxidizing reaction seems not to proceed perfectly. Secondly, in the case of liquid sample injection, the on-column injection method was used in this study, while the split injection method was used in the reported studies. In general, the on-column injection method has a smaller uncertainty for repeatability of injected sample volume than that for the split injection method. Therefore, the uncertainties for our measurement results were smaller. The sample preparation schemes may also be the reason. In this study, the uncertainties for the obtained results included the uncertainties for the sample preparation schemes. On the other hand, there is no description of the precision of sample preparations in the studies reported by other groups, so the sample preparations may not have been reliable.

## Conclusions

A determination method using post-column reaction GC-FID systems was used in the quantitative analysis of NOCs to validate the applicability of the system to the determination of the NOCs. The post-column reaction GC-FID systems used in this study do not require standard materials of the target NOCs, which are usually hazardous, and analysts do not have to handle NOC reagents during the analysis. The NOCs in acetone were determined by the post-column reaction GC-FID systems with calibration solution of hydrocarbons, and the determined concentrations were consistent with those calculated from the weight of each component within the uncertainties. It was made clear that the post-column reaction GC-FID systems are applicable for measuring the NOCs. The determination method using the post-column reaction GC-FID system is expected to be applicable for other volatile NOCs, and provides a safer analytical method for measurements of the NOCs.

## Supporting Information

Detailed preparation procedures for the testing mixtures and intermediate mixtures, purities (with uncertainties) of the raw

materials, and operating conditions for the two post-column reaction GC-FID systems are described in the Supporting Information. This material is available free of charge on the web at <http://www.jsac.or.jp/analsci/>.

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