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TITLE:

Nitrogen Compound Characterization in Fuels by Multidimensional Gas Chromatography

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KEYWORDS:

Two-dimensional gas chromatography, fuel, nitrogen specific detection, stability, nitrogen compounds, sample characterization

SUMMARY:

Here, we present a method utilizing two-dimensional gas chromatography and nitrogen chemiluminescence detection (GCxGC-NCD) to extensively characterize the different classes of nitrogen-containing compounds in diesel and jet fuels.

ABSTRACT:

Certain nitrogen-containing compounds can contribute to fuel instability during storage. Hence, detection and characterization of these compounds is crucial. There are significant challenges to overcome when measuring trace compounds in a complex matrix such as fuels. Background interferences and matrix effects can create limitations to routine analytical instrumentation, such as GC-MS. In order to facilitate specific and quantitative measurements of trace nitrogen compounds in fuels, a nitrogen-specific detector is ideal. In this method, a nitrogen chemiluminescence detector (NCD) is used to detect nitrogen compounds in fuels. NCD utilizes a nitrogen-specific reaction that does not involve the hydrocarbon background. Two-dimensional (GCxGC) gas chromatography is a powerful characterization technique as it provides superior separation capabilities to one-dimensional gas chromatography methods. When GCxGC is paired with a NCD, the problematic nitrogen compounds found in fuels can be extensively characterized without background interference. The method presented in this manuscript details the process for measuring different nitrogen-containing compound classes in fuels with little sample

preparation. Overall, this GCxGC-NCD method has been shown to be a valuable tool to enhance the understanding of the chemical composition of nitrogen-containing compounds in fuels and their impact on fuel stability. The % RSD for this method is <5% for intraday and <10% for interday analyses; the LOD is 1.7 ppm and the LOQ is 5.5 ppm.

INTRODUCTION:

Before use, fuels undergo extensive quality assurance and specification testing by refineries to verify that the fuel they are producing will not fail or cause equipment problems once disseminated. These specification tests include flash point verification, freeze point, storage stability, and many more. The storage stability tests are important as they determine if the fuels have a tendency to undergo degradation during storage, resulting in the formation of gums or particulates. There have been incidences in the past when F-76 diesel fuels have failed during storage even though they passed all specification tests[1]. These failures resulted in high concentrations of particulate matter in the fuels that could be detrimental to equipment such as fuel pumps. The extensive research investigation that followed this discovery suggested that there is a causal relationship between certain types of nitrogen compounds and the particulate formation[2-5]. However, many of the techniques used to measure nitrogen content are strictly qualitative, require extensive sample preparation, and provide little information on the identity of the suspect nitrogen compounds. The method described herein is a two-dimensional GC (GCxGC) method paired with a nitrogen chemiluminescence detector (NCD) that was developed for the purpose of characterizing and quanitifying trace nitrogen compounds in diesel and jet fuels.

Gas chromatography is used extensively in petroleum analyses and there are over sixty published ASTM petroleum methods associated with the technique. A wide range of detectors are combined with gas chromatography such as mass spectrometry (MS, ASTM D2789[6], D5769[7]), Fourier-transform infrared spectroscopy (FTIR, D5986[8]), vacuum ultraviolet spectroscopy (VUV, D8071[9]), flame ionization detector (FID, D7423[10]), and chemiluminesence detectors (D5504[11], D7807[12], D4629-17[13]). All these methods can provide significant compositional information about a fuel product. Since fuels are complex sample matrices, gas chromatography enhances compositional analysis by separating out sample compounds based on boiling point, polarity, and other interactions with the column.

To further this separation ability, two-dimensional gas chromatography (GCxGC) methods can be utilized to provide compositional maps by using sequential columns with orthogonal column chemistries. Separation of compounds occur both by polarity and boiling point, which is a comprehensive means to isolate fuel constituents. Although it is possible to analyze nitrogen-containing compounds with GCxGC-MS, the trace concentration of the nitrogen compounds within the complex sample inhibits identification[14]. Liquid-liquid phase extractions have been attempted in order to use GC-MS techniques; however, it was found that the extractions are incomplete and exclude important nitrogen compounds[15]. Additionally, others have used solid phase extraction to enhance the nitrogen signal while reducing the potential for the fuel sample matrix interference[16]. However, this technique has been found to irreversible retail certain nitrogen species, especially low molecular weight nitrogen-bearing species.

The nitrogen chemiluminescence detector (NCD) is a nitrogen-specific detector and has been successfully used for fuel analyses[17-19]. It utilizes a combustion reaction of nitrogen-containing compounds, the formation of nitric oxide (NO), and a reaction with ozone (see Equations 1 & 2)[20]. This is accomplished in a quartz reaction tube that contains a platinum catalyst and is heated to 900 °C in the presence of oxygen gas.

The photons emitted from this reaction are measured with a photomultiplier tube. This detector has a linear and equimolar response to all nitrogen-containing compounds because all nitrogen-containing compounds are converted to NO. It is also not prone to matrix effects because other compounds in the sample are converted to non-chemiluminescence species (CO_2 and H_2O) during the conversion step of the reaction (equation 1). Thus, it is an ideal method for measuring nitrogen compounds in a complex matrix such as fuels.

$$R\text{-}N + O_2 \longrightarrow NO + CO_2 + H_2O \qquad \text{(Equation 1)}$$

$$NO + O_3 \longrightarrow NO_2 + O_2 + h\nu \qquad \text{(Equation 2)}$$

The equimolar response of this detector is important for nitrogen compound quantitation in fuels because the complex nature of fuels does not allow for calibration of each nitrogen analyte. The selectivity of this detector facilitates the detection of trace nitrogen compounds even with a complex hydrocarbon background.

PROTOCOL:

CAUTION: Please consult relevant safety data sheets (SDS) of all compounds before use. Appropriate safety practices are recommended. All work should be performed while wearing personal protective equipment such as gloves, safety glasses, lab coat, long pants, and closed-toed shoes. All standard and sample preparations should be done in a ventilated hood.

1. Preparation of standards

1.1 Prepare a 5,000 mg/kg (ppm) solution of carbazole (calibration standard, minimum of 98% purity) by placing 0.050 g in a vial and bring the total mass of each solution to 10.000 g with isopropyl alcohol. Cap the vial immediately to prevent loss of isopropyl alcohol. This is the calibration stock solution.

1.2 Prepare a carbazole solution with 100 ppm nitrogen content by diluting 1.194 mL of stock solution to 5 mL with isopropyl alcohol. This is designated as “100 ppm nitrogen carbazole” and is used to create the calibration standards.

NOTE: The concentrations of the calibration standards indicate the concentration of nitrogen in the standard, not the carbazole concentration

1.3 Prepare the following calibration standards by serial dilution:

20 ppm nitrogen carbazole
10 ppm nitrogen carbazole
5 ppm nitrogen carbazole
1 ppm nitrogen carbazole
0.5 ppm nitrogen carbazole
0.025 ppm nitrogen carbazole

1.4 Put 1 mL of the calibration standards into separate GC vials (6 vials total).

1.5 Prepare individual 10 ppm solutions of each standard compounds listed in **Table 1** in isopropyl alcohol. Place 1 mL of each standard solution in separate GC vials (10 vials total).

NOTE: The standard compounds listed in **Table 1** will be used to classify the unknown nitrogen compounds as 'light nitrogen compounds', 'basic nitrogen compounds', or 'non-basic nitrogen compounds'.

[Place **Table 1** here]

2. Sample preparation

2.1 For diesel fuels: In a GC vial, add 250 µL of fuel sample and 750 µL of isopropyl alcohol.

2.2 For jet fuels: In a GC vial, add 750 µL of fuel sample and 250 µL of isopropyl alcohol.

NOTE: If the total nitrogen concentration of either diesel or jet fuel falls below the calibration curve (0.025 ppm nitrogen) when diluted as instructed above, do not dilute. If the nitrogen concentration in a specific nitrogen group in either diesel or jet fuel falls above the calibration curve (20 ppm nitrogen), dilute sample further.

3. Instrument setup

3.1 Instrument configuration

3.1.1 Auto-sampler: Ensure that the autosampler tray and tower are installed with a splitless inlet and wash vials in place.

3.1.2 Nitrogen Chemiluminescence Detector: Ensure that the nitrogen chemiluminescence detector is installed with the appropriate gas lines (i.e., helium and hydrogen). A hydrogen generator can be utilized instead of a tank, if available.

3.1.3 Duel Loop Thermal Modulator: Ensure that the duel loop thermal modulator is installed and aligned properly so that the column loop will be centered between the cold and hot jet flows during modulation.

3.2 Column installation

3.2.1 Ensure that the instrument is in maintenance mode (i.e., all burners and gas flows are turned off).

3.2.2 Insert the 30 m primary column into the GC oven and connect to the splitless inlet.

3.2.3 Measure and cut 2.75 m of the secondary column. Put a mark on the secondary column at 0.375 m and 1.375 m using a white-out pen.

3.2.4 Place the secondary column into the Zoex modulator column holder and use the marks as guides for creating a 1 m loop within the holder for modulation.

3.2.5 Connect the shorter end of the secondary column to the primary column using a micro-union. Check for a successful connection by turning on the gas flow and inserting the open end of the column into a vial of methanol. A successful connection is confirmed by the presence of bubbles.

3.2.6 Place the column holder into the modulator and adjust the loops as necessary so that the loops line up properly with the cold and hot jets, as pictured in **Figure 1**.

3.2.7 Insert the other end of the column into the NCD burner. Then turn on all burners and gas flows to ensure there are no leaks.

3.2.8 Turn the oven on at the maximum temperature limit for a minimum of 2 h in order to bake-out the columns. Once completed, verify that there are no new leaks. Then, cool the oven.

[Place **Figure 1** here]

3.3 Method parameters

3.3.1 Using the computer software, set the instrument to the parameters listed in **Table 2**.

[Place **Table 2** here]

3.3.2 Set the initial oven temperature to 60 °C with a ramp rate of 5 °C/min to 160 °C, and then change the ramp rate to 4 °C until 300 °C. The total run time is 55 minutes per sample.

3.3.3 Set the hot jet temperature to be 100 °C higher than the oven temperature at any point in time. Thus: set the initial hot jet temperature to 160 °C with a ramp rate of 5 °C/min to 260 °C, and then change the ramp rate to 4 °C until 400 °C.

3.3.4 Set the ancillary liquid nitrogen Dewar connected to the GC to stay between 20% and 30% full during the run.

4. Instrument calibration

4.1 Place the GC sample vials containing the prepared carbazole standards and load the previously configured method into the GC software.

4.2 Create a sequence that aliquots the blank (isopropyl alcohol) at the beginning followed by the prepared carbazole standards by increasing concentration.

4.3 Verify that the liquid nitrogen Dewar is between 20-30% full and all instrument parameters are in “ready” mode. Start the sequence.

4.4 Once the calibration standard set analysis is completed, use the GCImage software to load each chromatogram, background correct, and detect each carbazole peak or blob.

NOTE: In GCImage, the detected peaks within the chromatogram are termed as “blobs” by the software.

4.5 In a spreadsheet program, plot the response (blob volume) against the nitrogen concentration (ppm) of each calibration standard to create a calibration curve (see **Figure 2**). The trend line of the curve should have $R^2 \geq 0.99$.

[Place **Figure 2** here]

5. Sample analysis

5.1 Place the GC sample vials in the autosampler tray and load the previously configured method.

5.2 Create a sequence that has a blank (isopropyl alcohol) at the beginning and then every 5 subsequent samples to limit any build-up of fuel within the columns.

5.3 Verify that enough liquid nitrogen is available in the modulator’s Dewar and that all instrument parameters are in “ready” mode. Then, start the sequence.

6. Data analysis

6.1 Open the chromatogram in the GCImage software for data analysis and perform a background correction

6.2 Detect blobs using the following filter parameters:
    Minimum area = 25
    Minimum volume = 0

Minimum peak = 25

NOTE: These parameters are subject to change based on instrument response or sample matrix.

6.3 Use the GCImage template function to create or load a template to group nitrogen compound classes based on the elution times of the known standards (see **Table 1**).

NOTE: Further explanation of template usage can be found in the representative results and **Figure 8**.

6.4 Once the compounds have been grouped, export the “blob set table” into a spreadsheet program. Use the sum the volume of all blobs/peaks within each compound class group, and the calibration equation determined in Section 4.4 to calculate the concentration in ppm for the nitrogen compounds in each group.

6.5 If desired, use the following density calculations to correct for differences in the injection volume of sample vs. standards for quantitation:

100 ppm N in IPA, 1 µL injection volume

$$\left(\frac{100\,ng\,N}{mg\,IPA}\right)\left(\frac{786\,mg\,IPA}{mL}\right)(0.001\,mL\,injected) = 78.6\,ng\,N\,injected \qquad \text{(Equation 3)}$$

100 ppm N in low density jet

$$\left(\frac{100\,ng\,N}{mg\,jet}\right)\left(\frac{775\,mg\,jet}{mL}\right)(0.001\,mL\,injected) = 77.5\,ng\,N\,injected\ \text{(-1.3\%)*} \qquad \text{(Equation 4)}$$

100 ppm N in F-76

$$\left(\frac{100\,ng\,N}{mg\,F76}\right)\left(\frac{876\,mg\,F76}{mL}\right)(0.001\,mL\,injected) = 87.6\,ng\,N\,injected\ \text{(+10\%)*} \qquad \text{(Equation 5)}$$

100 ppm N in heavy MGO

$$\left(\frac{100\,ng\,N}{mg\,MGO}\right)\left(\frac{890\,mg\,MGO}{mL}\right)(0.001\,mL\,injected) = 89.0\,ng\,N\,injected\ \text{(+13\%)*} \qquad \text{(Equation 6)}$$

NOTE: *percent difference between the ng N injected in the sample matrix vs. the standard matrix

6.6 Sum all nitrogen content in each compound class to obtain the total nitrogen content of the sample, if desired. If the total nitrogen content is determined to be above 150 ppm nitrogen or a compound class bin is outside of the calibration range, dilute the sample further for analysis. Compare these results with total nitrogen content as determined by ASTM D4629[13] for quantification verification.

REPRESENTATIVE RESULTS:

The nitrogen-containing compound, carbazole, was used in this method as the calibration standard. Carbazole elutes at approximately 33 min from the primary column and at 2 s from the secondary column. These elution times will vary slightly depending on the exact column length and instrumentation. In order to obtain a proper calibration curve and, subsequently, good quantitation of nitrogen compounds within a sample, the calibration peaks should not be overloaded nor have any nitrogen contaminants. The primary and secondary column chromatograms of the carbazole calibration standard containing 0.025 ppm N are shown in **Figure 3**. There is not any tailing and the standard response is outside of the noise.

[Place **Figure 3** here]

Figure 4 is an example of a GCxGC-NCD chromatogram with a carbazole standard and the resulting blob table. As can be seen, there are two detected blobs that are not within the carbazole elution time, and they have been excluded from the blob table. Extraneous peaks or blobs should not be included in the calibration curve.

[Place **Figure 4** here]

Figure 5 illustrates a typical chromatogram obtained by using this method on a diesel fuel sample and **Figure 6** is a typical chromatogram of a jet fuel sample. Typically, jet fuel has fewer nitrogen compounds at lower concentrations than a diesel fuel, which can be clearly seen when comparing the two chromatograms. The peaks or “blobs” in these chromatograms are oval-shaped (little-to-no ‘streaking’ or too much retention on either column) and are easily distinguished from each other. It is clear that different classes of nitrogen compounds are present in diesel fuel when compared to jet fuel.

[Place **Figure 5** here]

[Place **Figure 6** here]

In contrast to the previous examples, **Figure 7** illustrates two failed sample measurements. The image on the left occurs when the modulation time is incorrect for the oven temperature, consequently resulting to wrap-around in the column. The solution to this type of failure is to either increase the modulation time or increase the temperature of the oven. The chromatogram on the right illustrates a “streaking” effect of the blobs. This occurs when the compounds are retained on the sample for too long and it destroys any compound separation. From experience, this tends to be caused by a build-up of compounds within the column. This problem can be repaired by running multiple blanks and “burning out” the column by increasing the temperature of the oven to 300 °C and allowing it to sit for several hours at that temperature.

[Place **Figure 7** here]

Standards (as listed in **Table 1**) can be utilized to determine the groups associated with each nitrogen compound class. An example of these standard groups can be seen in **Figure 8**. The retention times of the standards may differ slightly on different instrumentation or different column sets. Hence, it is imperative to run the standards each time an instrument parameter is changed.

[Place **Figure 8** here]

A template can be created in GCImage to separate the nitrogen compounds found in fuel by different nitrogen classes. The template should be built from the elution times determined by the standards and then overlaid on each fuel chromatogram. **Figure 9** is a representation of a template with the three groups as determined by the standard elution times. Once the template is overlaid, the blob set table will indicate the number of blobs and the total volume within each classified group.

[Place **Figure 9** here]

The response factor from the calibration curve should then be used to calculate the concentration of nitrogen compounds within each nitrogen class. **Figure 10** depicts the concentration in ppm for nitrogen compounds detected in each of the three classes for a batch of diesel fuel samples.

[Place **Figure 10** here]

FIGURE AND TABLE LEGENDS:

Table 1. Nitrogen standards and their elution classification groups

Table 2. Instrument parameters

Figure 1. Schematic representation of the GCxGC-NCD instrumentation. This figure has been reprinted from Deese et al.

Figure 2. Example GCxGC-NCD carbazole calibration curve

Figure 3. Representative chromatograms of 0.025 ppm N carbazole calibration standard on the primary (left) and secondary (right) columns.

Figure 4. Representative GCxGC-NCD chromatogram of a carbazole standard diluted with isopropyl alcohol. The extraneous peaks are circled in yellow.

Figure 5. Representative GCxGC-NCD chromatogram containing the nitrogen compounds found in a diesel fuel.

Figure 6. Representative GCxGC-NCD chromatogram containing the nitrogen compounds found in a jet fuel.

Figure 7. Representation of failed chromatograms. Wrap around caused by incorrect modulation time (left) and peak degradation caused by sample retention on the column (right).

Figure 8. An example of the retention times of the standards listed in Table 1.

Figure 9. Representative GCxGC-NCD chromatogram with overlaid template and blob set table

Figure 10. Representative results of the nitrogen concentration (ppm) in diesel fuels by group.

Supplementary Table 1. The intraday and interday repeatability of total nitrogen concentration by GCxGC-NCD for four fuels.

DISCUSSION:

The purpose of this method is to provide detailed information on the nitrogen content of diesel and jet fuels without extensive sample preparation such as liquid extractions. This is achieved by pairing a two dimensional GC system (GCxGC) with a nitrogen-specific detector (nitrogen chemiluminescence detector, NCD). The GCxGC provides significant separation of the compounds relative to traditional one-dimensional GC. The NCD provides trace nitrogen compound detection without any background interferences. Other nitrogen-specific detectors that have been used in the past, such as a nitrogen phosphorus detector (NPD), are interfered by the fuel's hydrocarbon matrix. In contrast, this method has little-to-no matrix interferences.

This GCxGC method uses a reverse-phase (polar-to-nonpolar) column setup so the compounds in the first dimension are separated by polarity while in the second dimension they are separated by boiling point. The second dimension separation is controlled by a thermal modulator that re-concentrates compounds via cryo-focusing and then separates the compounds further. The secondary column within the modulator must be accurately placed in order to achieve optimal separation. If the column loop is not centered between the hot and cold jet, the peaks will not have the proper shape or elute correctly. Furthermore, helium is used as the carrier gas for this system. Although hydrogen gas could be used as a carrier gas, there is a possibility that it can create active sites, which will interact with the nitrogen compounds. In order to completely eliminate that possibility, helium is highly recommended.

Because of the trace nature of the nitrogen compounds found in these fuels, mass spectrometry characterization is difficult to obtain. The most effective way to identify the nitrogen compound classes with this system is by injecting a variety of known nitrogen-containing compounds and creating a nitrogen class template based on these standards (see **Table 1**). The elution times of these compounds may vary slightly depending on the instrument used. Thus, it is imperative that the standard set is measured on each instrument and a unique template is created. This template can then be utilized for the fuel samples in order to characterize the classes of nitrogen compounds in a fuel and to provide quantitative information.

The ideal method for quantification of these compounds is to sum the total blob volume within each class, use the calibration equation to calculate the concentration of nitrogen per class, and then sum the class content to obtain the total nitrogen concentration. The repeatability of these measurements for analyses on both the same day and over different days has been found to be

< 20% RSD (see **Supplementary Files**). The highest limit of detection (LOD) and limit of quantitation (LOQ) has been found to be 1.7 ppm and 5.5 ppm, respectively (see **Supplementary Files**).

To the best of our knowledge, the purpose of the method detailed is to provide significant characterization of the classes of nitrogen compounds in diesel and jet fuels. Other nitrogen characterization methods require the use of liquid extractions (which have been found to exclude imperative nitrogen compounds) and detection schemes that have significant matrix interferences. Both jet and diesel samples can be measured by using the same method and instrument configuration, the only difference is the extent of dilution of the samples before measurement. There are current efforts underway to use this GCxGC-NCD method as a way of further characterizing fuels (in addition to the published ASTM methods) in order to determine and predict fuel quality. This characterization project includes increasing the number of nitrogen standards utilized to create a reliable template to enhance the chemical compositional analysis of fuels containing nitrogen compounds, which will further refine the understanding of compounds that are detrimental to fuels in long term storage.

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DISCLOSURES:

The authors have nothing to disclose.

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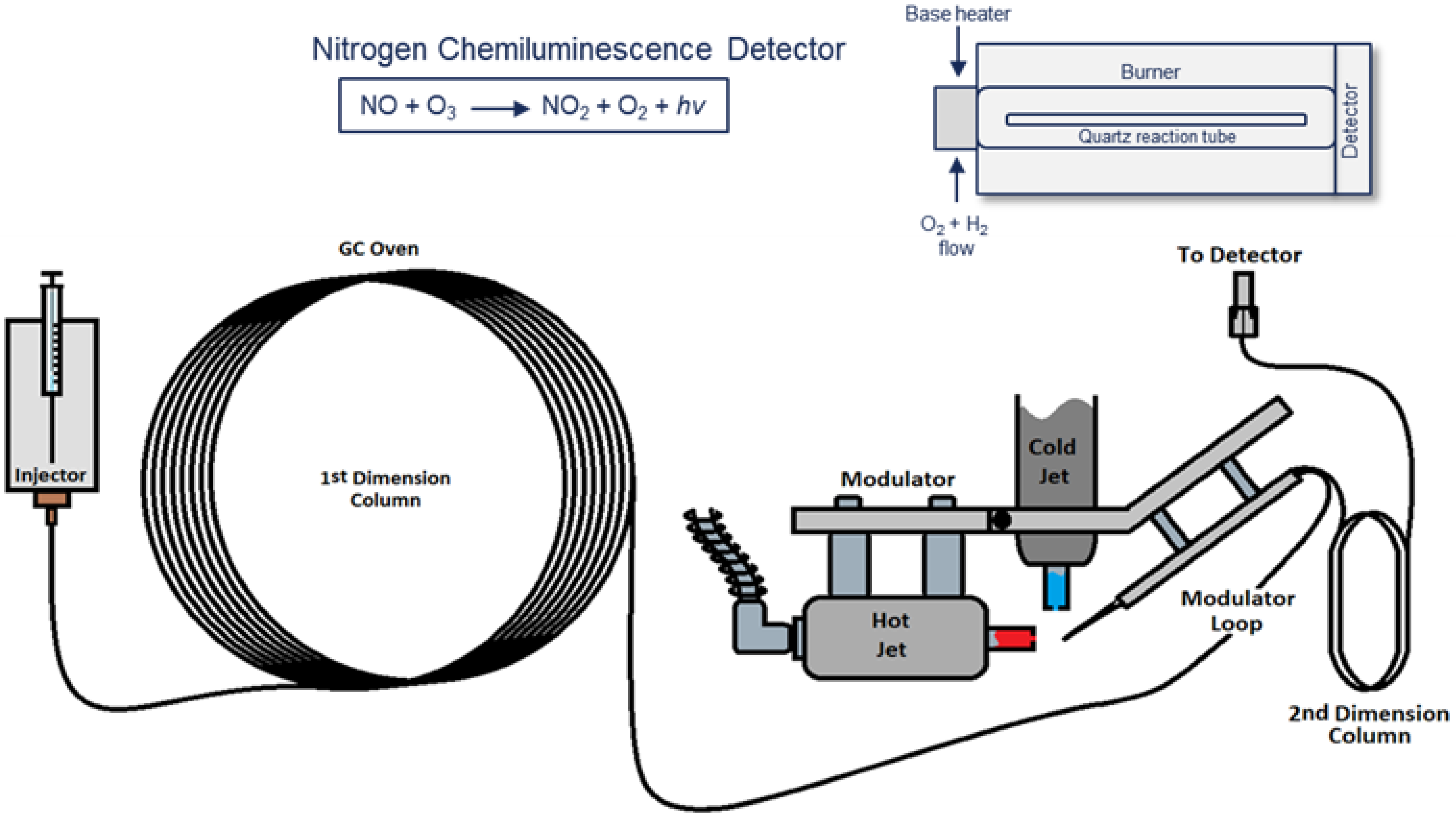

Figure 2

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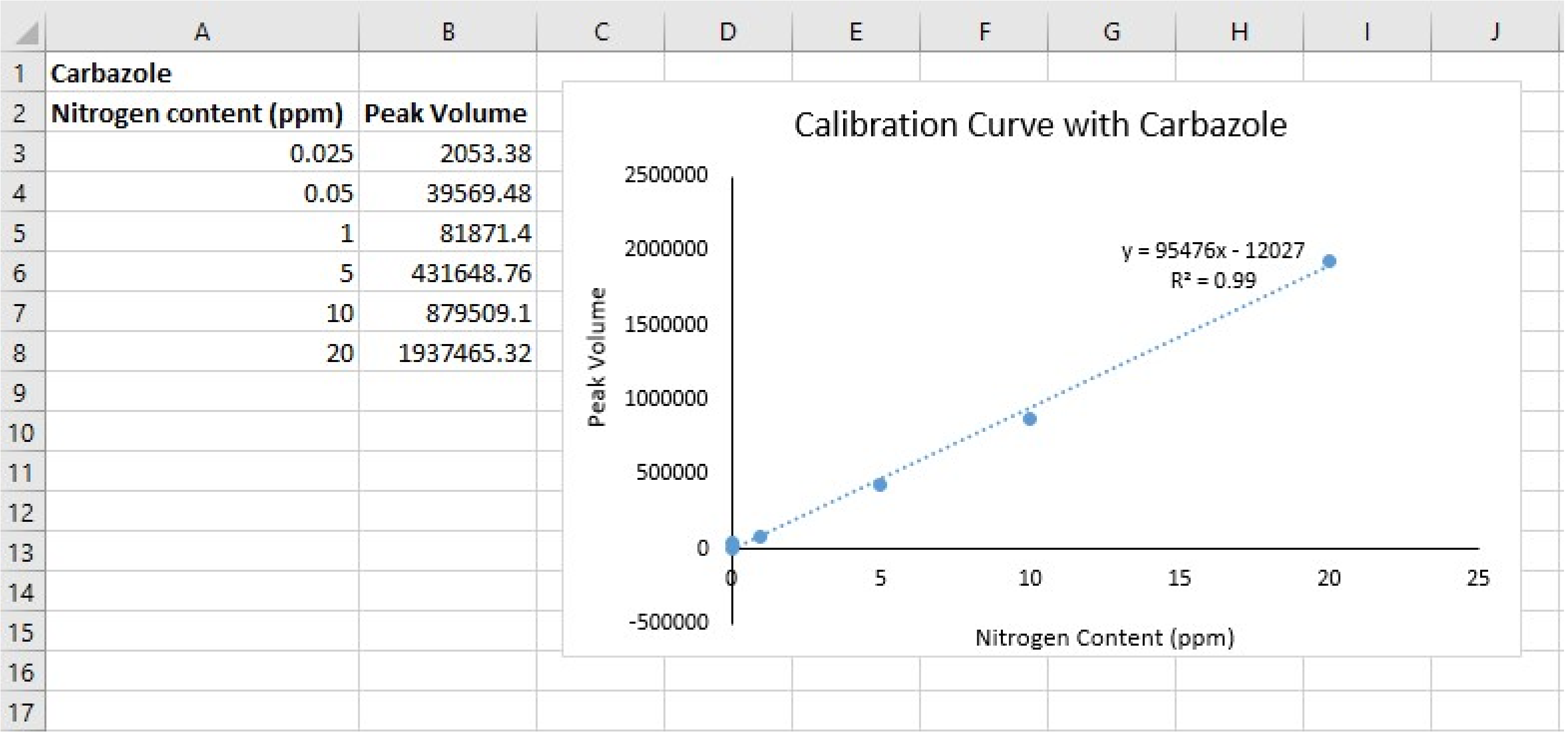

	A	B
1	**Carbazole**	
2	**Nitrogen content (ppm)**	**Peak Volume**
3	0.025	2053.38
4	0.05	39569.48
5	1	81871.4
6	5	431648.76
7	10	879509.1
8	20	1937465.32

Figure 3

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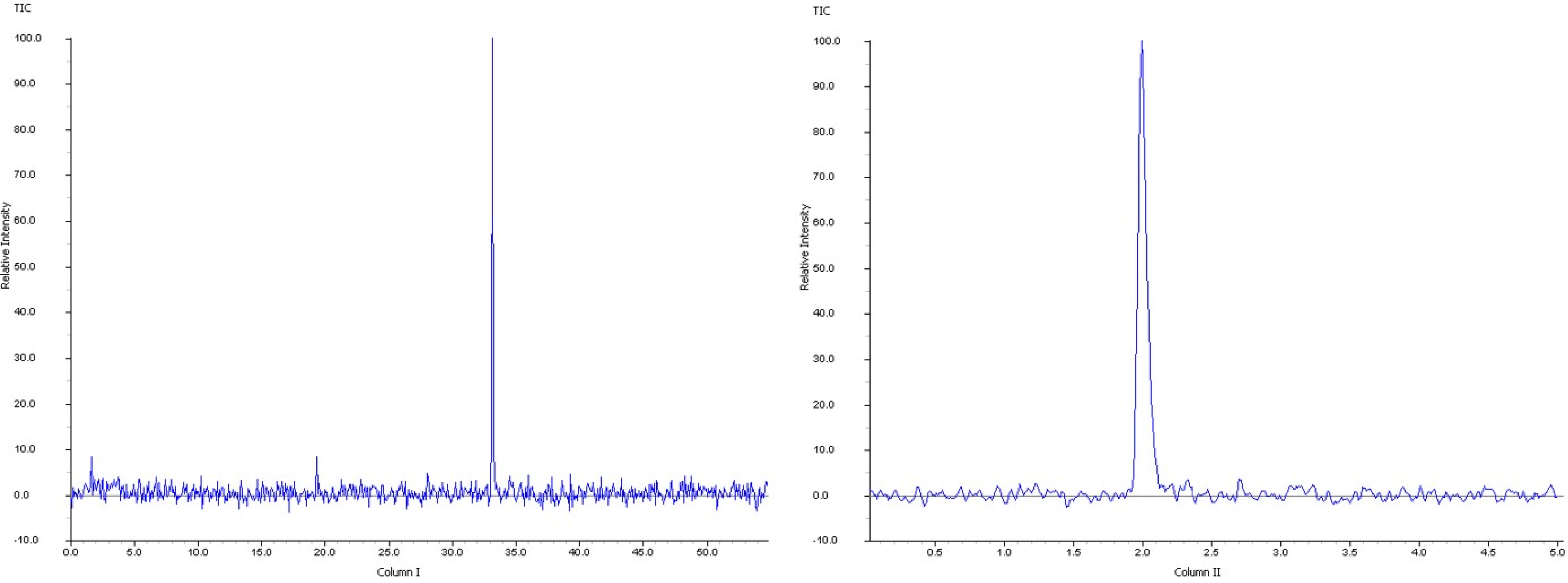

Figure 4

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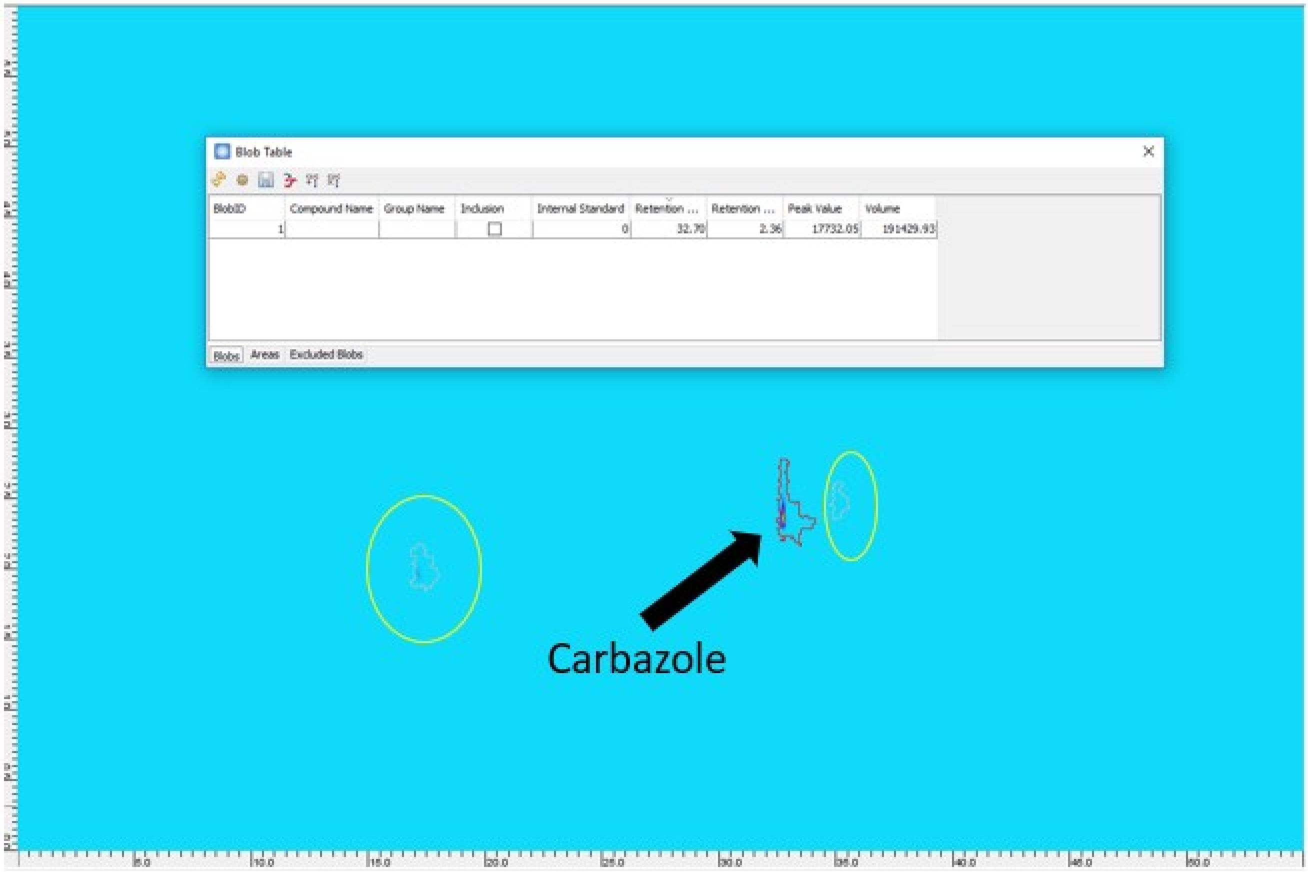

BlobID	Compound Name	Group Name	Inclusion	Internal Standard	Retention ...	Retention ...	Peak Value	Volume
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Figure 5

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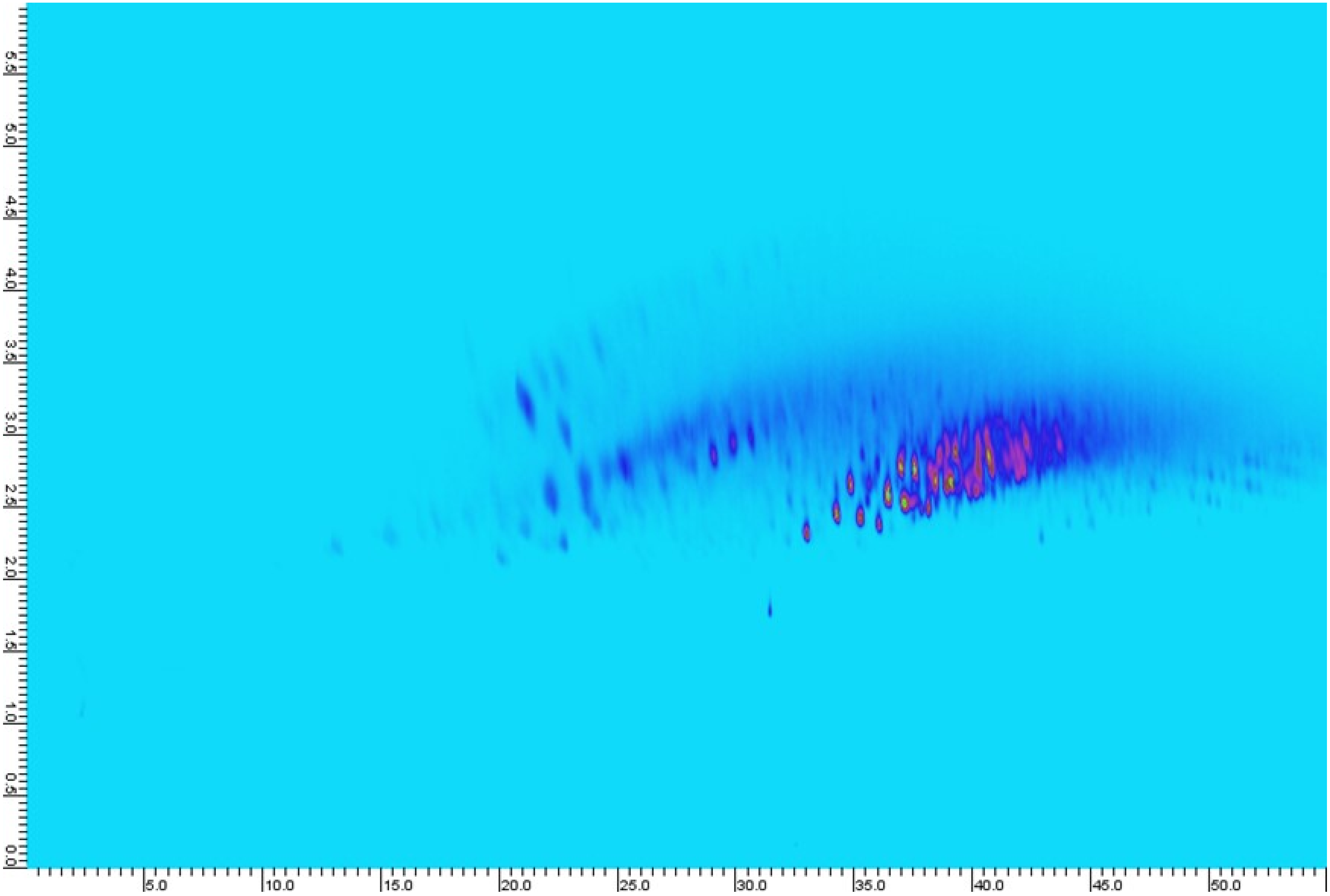

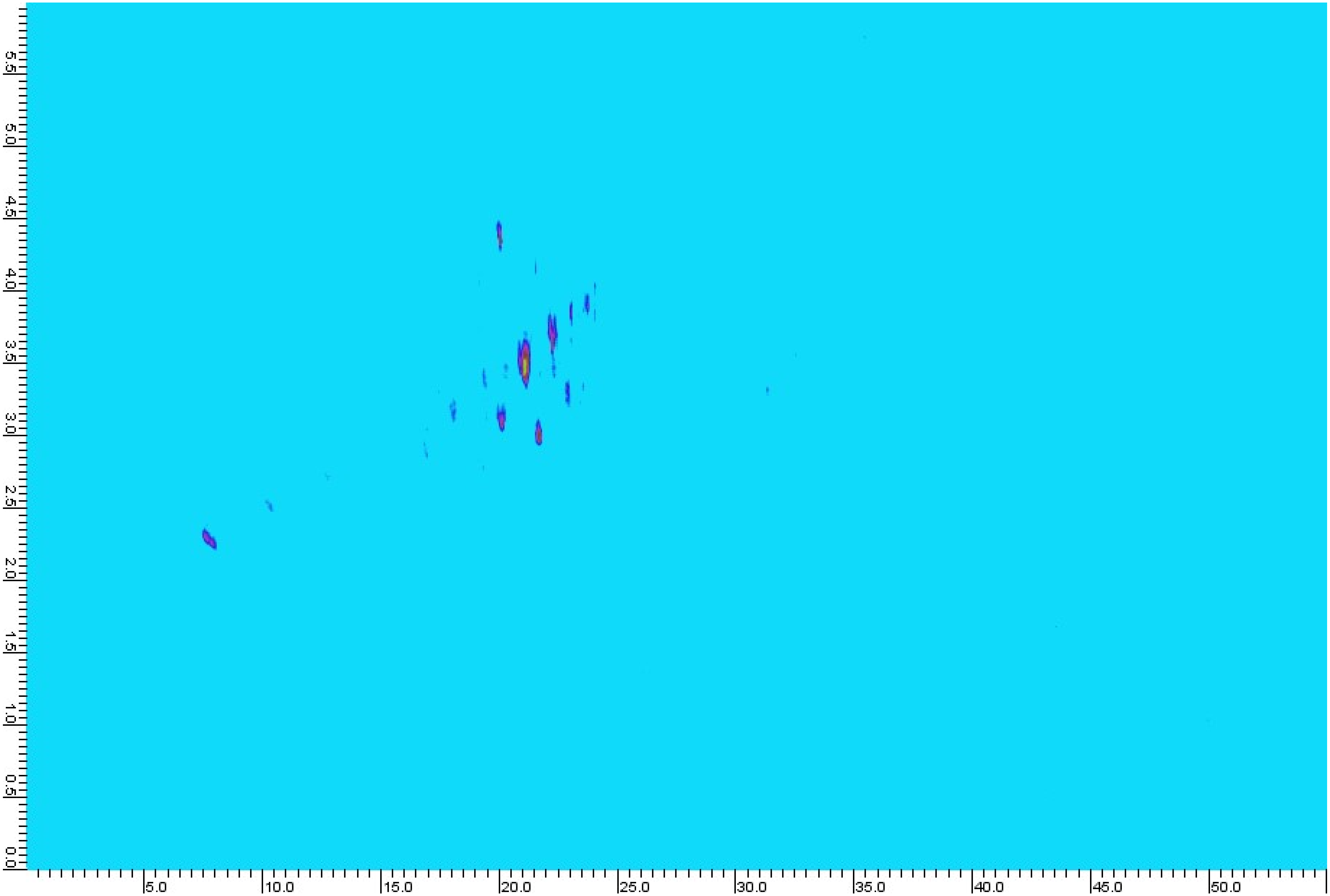
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Figure 7

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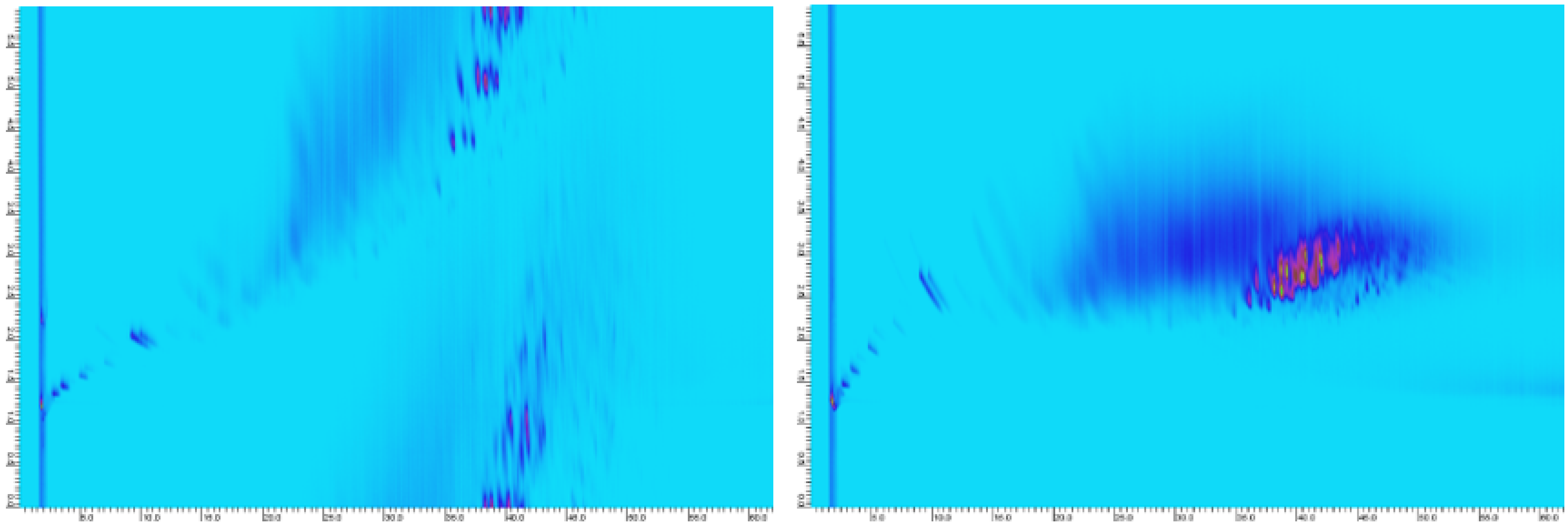

Figure 8

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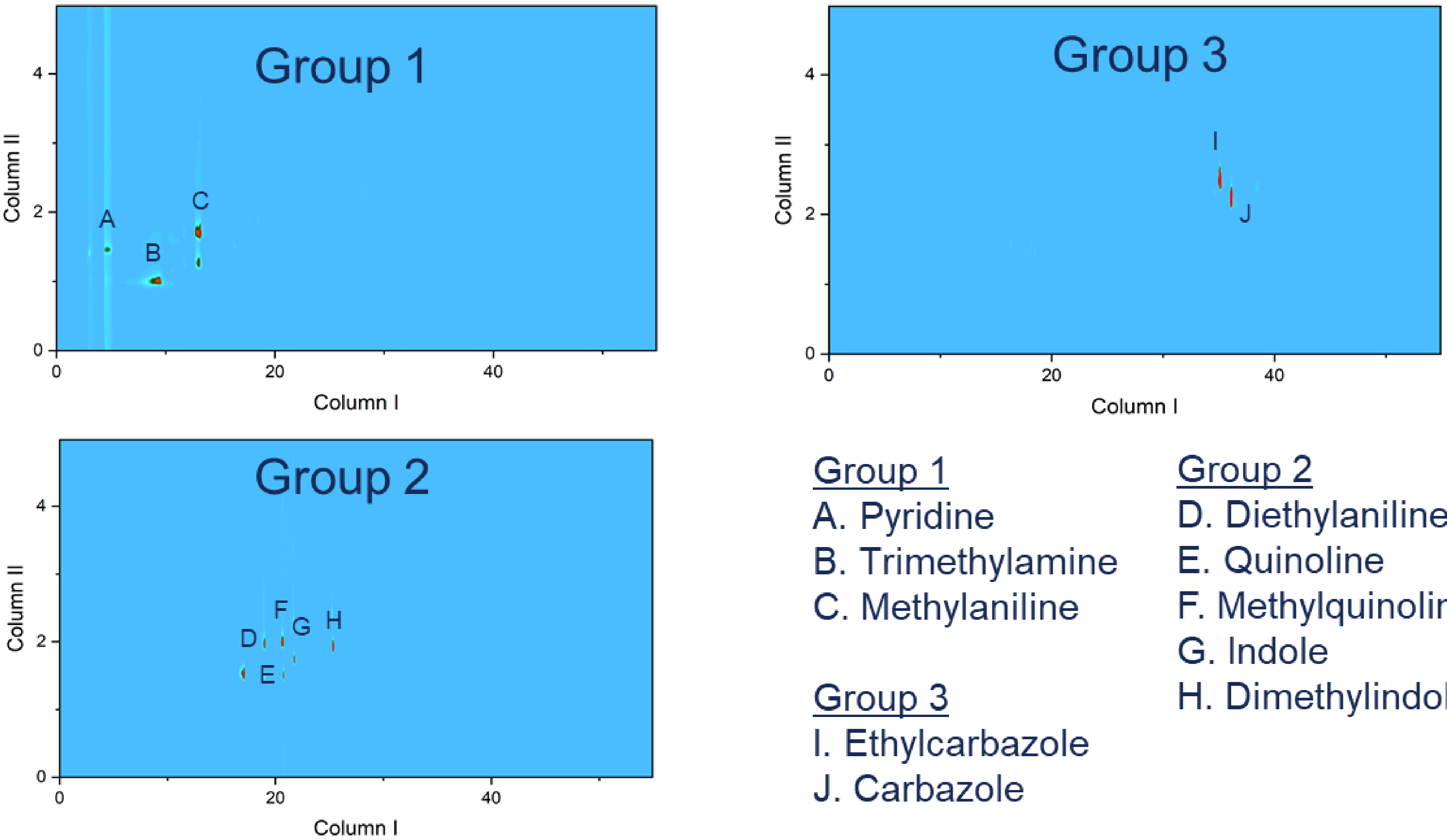

Group 1
A. Pyridine
B. Trimethylamine
C. Methylaniline

Group 3
I. Ethylcarbazole
J. Carbazole

Group 2
D. Diethylaniline
E. Quinoline
F. Methylquinoline
G. Indole
H. Dimethylindole

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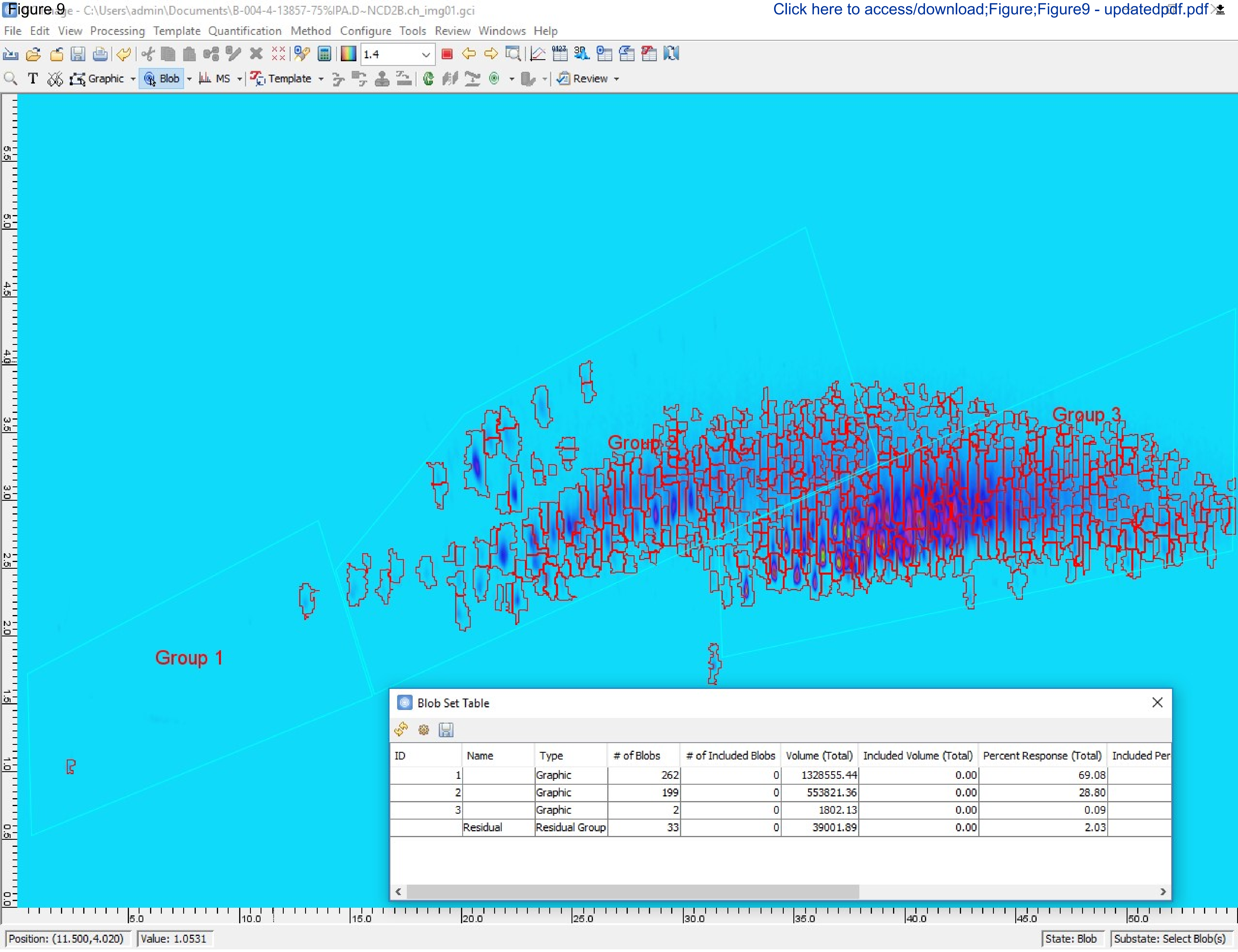
Figure 9



ID	Name	Type	# of Blobs	# of Included Blobs	Volume (Total)	Included Volume (Total)	Percent Response (Total)	Included Per
1		Graphic	262	0	1328555.44	0.00	69.08	
2		Graphic	199	0	553821.36	0.00	28.80	
3		Graphic	2	0	1802.13	0.00	0.09	
	Residual	Residual Group	33	0	39001.89	0.00	2.03	

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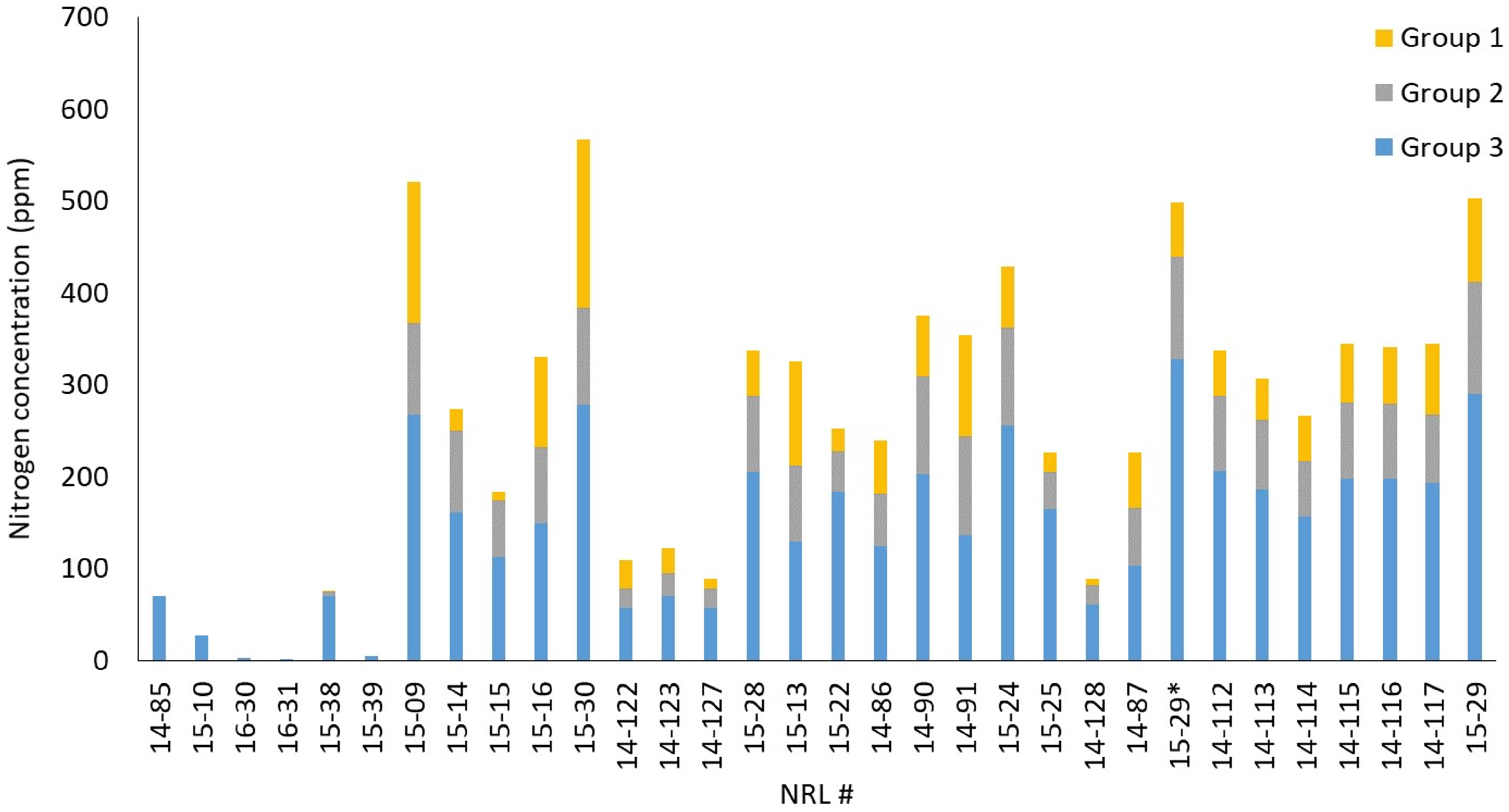

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Standard Compound	Elution Time Classification Group
Pyridine	Group 1 – light nitrogen compounds
Trimethylamine	Group 1 – light nitrogen compounds
Methylaniline	Group 1 – light nitrogen compounds
Quinoline	Group 2 – basic nitrogen compounds
Diethylaniline	Group 2 – basic nitrogen compounds
Methylquinoline	Group 2 – basic nitrogen compounds
Indole	Group 2 – basic nitrogen compounds
Dimethylindole	Group 2 – basic nitrogen compounds
Ethylcarbazole	Group 3 – Non-basic nitrogen compounds
Carbazole	Group 3 – Non-basic nitrogen compounds

Table 2

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Instrument Parameters

NCD	Nitrogen Base Temperature	280 °C
	Nitrogen Burner Temperature	900 °C
	Hydrogen flow rate	4 mL/min
	Oxidizer flow rate (O2)	8 mL/min
	Data collection rate	100 Hz
Inlet	Inlet Temperature	300 °C
	Inlet liner	Splitless
	Purge flow to split vent	15 mL/min
	Septum Purge Flow	3 mL/min
	Carrier gas	He
	Carrier gas flow rate	1.6 mL/min
	Syringe size	10 µL
	Injection volume	1 µL
Modulator	Modulation time	6000 ms
	Hot pulse duration	375 ms
Columns	Flow	1.6 mL/min
	Flow type	Constant flow

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Name of Material/ Equipment	**Company**	**Catalog Number**	**Comments/Description**
10 µL syringe	Agilent		gold series
180 µm x 0.18 µm Secondary Column	Restek	Rxi-1MS	nonpolar phase column, crossbond dimethyl polysiloxane
250 µm x 0.25 µm Primary Column	Restek	Rxi-17SilMS	midpolarity phase column
Autosampler tray and tower	Agilent	7963A	
Carbazole	Sigma	C5132	98%
Diethylaniline	Aldrich	185898	≥ 99%
Dimethylindole	Aldrich	D166006	97%
Duel Loop Thermal Modulator	Zoex Corporation	ZX-1	
Ethylcarbazole	Aldrich	E16600	97%
Gas chromatograph	Agilent	7890B	
GC vials	Restek	21142	
GCImage Software, Version 2.6	Zoex Corporation		
Indole	Aldrich	13408	≥ 99%
Isopropyl Alcohol	Fisher Scientific	A461-500	Purity 99.9%
Methylaniline	Aldrich	236233	≥ 99%
Methylquinoline	Aldrich	382493	99%
Nitrogen Chemiluminescence Detector	Agilent	8255	
Pyridine	Sigma-Aldrich	270970	anhydrous, 99.8%
Quinoline	Aldrich	241571	98%
Trimethylamine	Sigma-Aldrich	243205	anhydrous, ≥ 99%

Rebuttal Letter

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Mr. Benjamin Werth
Senior Science Editor
JOVE

25 November 2019

Dear Mr. Werth,

Thank you for the opportunity to revise our paper "Nitrogen Compound Characterization in Fuels by Multidimensional Gas Chromatography". We greatly appreciate the reviewer's comments as they were very helpful.

Below, I have included the reviewer comments and I have responded to them individually to indicate how each concern was addressed. The revisions have been approved by all authors. I have uploaded a revised manuscript.

Sincerely,

Rachel D. Deese

Editorial comments:
The manuscript has been modified and the updated manuscript, 60883_R0.docx, is attached and located in your Editorial Manager account. Please use the updated version to make your revisions.

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4. Please add a short description of a figure in Figure Legend for each figure.
5. Please do not abbreviate journal titles for references.
6. The superscript reference number is inserted immediately next to the word/group of words it applies to but before any punctuation.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:
Solid paper. Concise and clear. Useful method. The Abstract should include a quantitative summary, it did not. More use of the 2D separation space would have been welcome.

Major Concerns:
None

Minor Concerns:
Please see summary

Response: The statistics for the method has been added to the abstract.

Reviewer #2:

Minor Concerns:

First of all, the article is written in a very professional manner, with sufficient details for a fellow researcher to repeat the analysis. The information provided in the article can be used as a working method for analysis.
The following are comments:

1. The authors mention that they are injecting equal volumes of the standards and the samples, however, the resulting difference between the standard and the samples is not taken into consideration when calculating densities. The final concentration calculation will be directly affected by the difference in densities.

Response: Density calculations have been included to help provide quantitation correction for the different sample types.

2. The authors used nitrogen carbazole for the calibration of the instrument. Was the nitrogen detector response tested for the rest of the NCD compounds mentioned in the article? In other words, do all NCD compounds give a similar enough detector response that only the nitrogen carbazole compound can be used for calibration?

Response: The NCD provides equimolar responses due to the nature of the reaction. The response of nitrogen compounds have been tested with two other standard compounds, pyridine and quinoline, and it was found that all standards had similar response factors. Carbazole was chosen as the standard for calibration over the others because it is an ASTM standard for nitrogen analysis and has lower volatility than pyridine (which makes the standards more stable over time).

3. The authors do not reference similar, previous works that have been done, such as:
* Sep Sci. 2004 Apr;27(5-6):468-72.
Speciation of nitrogen-containing in diesel fuel by comprehensive two-dimensional gas chromatography.
Wang FC1, Robbins WK, Greaney MA. Dampen
* Talanta 198 (2019) 263-276
Comprehensive two-dimensional gas chromatography for the analysis of nitrogen-containing compounds in fossil fuels: A review Maria Elisabete Machado

Response: Thank you for these references. They have been added to the manuscript.

4. The statistical data for RSD%/inter-day and RSD%/intra-day is missing. In addition, the stability of the method is not clear. What is the sensitivity of the method for the detection and calculation of the compound? (e.g. limits of detection).

Response: These statistics have been updated in the manuscript and the supplementary file

5. We believe the %RSD calculation in table 1 for the samples is incorrect. It should be:
RSD(%)=stdev(range)/average(range)*100%

Response: The %RSD information has been updated in the manuscript and the supplementary file

6. It is recommended to add at least one 2D chromatogram, so the resulting detector signal can be observed. One wonders how symmetric the peaks are for the lower concentration standards that are used in the method.

Response: Chromatograms have been added to illustrate the signal for the 0.025 ppm N carbazole standard (please see the new Figure 3).

7. The authors do not provide sufficient information with regards to the instrument which was used for method development. It is a crucial piece of information that should be included in the article.

Response: As per JoVE article guidelines – we did not include specific instrumentation information in the article but it is included in the Table of Materials. “Avoid the use of commercial language, including

™/®/© symbols or company brand names before/after an instrument or reagent. Cite these in the **Table of Materials** instead. "

8. The authors mentioned using the software GCImage for their calculations. Is it a commercial program? If so, the authors should mention the software developer and version number.

Response: As per JoVE article guidelines – we did not include specific instrumentation or software information in the article but it is included in the Table of Materials. "Avoid the use of commercial language, including ™/®/© symbols or company brand names before/after an instrument or reagent. Cite these in the **Table of Materials** instead. "

Reviewer #3:

This manuscript describes a technique for using GCxGC with an NCD detector to quantify nitrogen species classes in jet and diesel fuels. The paper is well-written and does a good job in describing the utility of the method and the details of the procedures. A number of minor issues need to be addressed before the paper is published.

Page 1, lines 41/42. The authors state "In order to measure trace nitrogen compounds in fuels, a nitrogen-specific detector is required as otherwise these trace compounds are impossible to detect from the background." This is too broad of a generalization as other methods that do not utilize a nitrogen-specific detector are also able to detect nitrogen species in fuel matrices, including ESI-MS and SPE followed by GC methods.

Response: This wording has been removed from the abstract as per the suggestion.

Page 2, line 83. "multidimensional gas chromatography (GCxGC)" would be better stated "two-dimensional gas chromatography (GCxGC)"

Response: This has been changed accordingly.

Page 2, line 88. Change "the complex sample attenuates identification" to "the complex sample inhibits identification" as attenuation does not appear to be the correct word in this context.

Response: This has been changed accordingly.

Page 2, line 88. "Liquid-liquid extractions …" are addressed here but what about the use of solid-phase extraction for isolating and concentrating these species?

Response: A full discussion of alternative sample preparation methods was not the focus of this article however we have expanded the discussion to a reference on GCxGC NCD with solid phase extraction.

Page 3, lines 92-94. The description of the NCD detector is perfunctory and unclear. What combustion reactions create NO from the N-species? How is it insured that these go to completion?

Response: This discussion has been expanded to clarify the combustion reactions and method of detection.

Page 6, lines 224-225 and lines 246-247. These sections warn about making sure the "liquid nitrogen dewar is between 20% and 30% full" but as other labs will have varying size liquid nitrogen dewars, indicating a percentage is not helpful. Perhaps state that one should "assure that sufficient liquid nitrogen is available for the runs to be performed"?

Response: This has been changed as suggested

Page 6, lines 261-262. The description of creation of a template for the nitrogen species classes is vague here - variance in the template between labs will affect the species class concentrations obtained. The template is described in more detail later (page 9-10) - perhaps reference this discussion here so the reader knows about it?

Response: A reference to the discussion has been added as suggested

Page 10, line 410. Liquid extractions are discussed but solid-phase extractions can also be useful.

Response: See earlier response

Figure 2. The calibration curve is shown in nanograms/mL which is approximately ppb, but the manuscript refers to a ppm range. As ppm is discussed in the manuscript, the figure should show ppm rather than ng/mL.

Response: This figure has been updated to reflect the correct units.

Figure 9. The calibration curve is performed up to 20 ppm nitrogen, but the results shown in Figure 9 cover the range up to 600 ppm. Ordinarily the cal curve should cover the total range of the analysis. Is the lower range of the calibration curve because it is performed on a single blob (compound) and multiple blobs (species) are added up to create the total N concentration for a species class? If this is true, the paper should address the selection of the calibration curve range and how it depends on the range of individual species concentrations to be found in the fuel.

Response: We have found that the linearity of the calibration is compromised above 150 ppm total nitrogen. To counter this the sample is diluted to achieve a total response under 150 ppm then each compound class is subjected to the 20 ppm calibration curve. The article has been undated to provide clarification.

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Supplementary Files

Supplementary Table 1. The intraday and interday repeatability of total nitrogen concentration by GCxGC-NCD for four fuels.

	Intraday			**Interday**		
Fuel	Average N concentration (ppm)	Standard deviation	% RSD	Average N concentration (ppm)	Standard deviation	% RSD
1	376.19	± 0.20	0.05	374.87	± 22.93	6.12
2	36.04	± 0.11	0.31	39.05	± 2.19	5.6
3	2.91	± 0.04	1.48	2.6	± 0.20	7.85
4	0.59	± 0.02	3.26	0.44	± 0.07	15.7

Limit of Detection and Limit of Quanititation

$$LOD = \frac{3\ x\ (standard\ deviation\ of\ blob\ volume\ for\ 25\ ppb)}{slope\ of\ the\ calibration\ curve} \qquad \text{(Equation S1)}$$

$$LOQ = \frac{10\ x\ (standard\ deviation\ of\ blob\ volume\ for\ 25\ ppb)}{slope\ of\ the\ calibration\ curve} \qquad \text{(Equation S2)}$$

LOD

Trial 1: 1.43

Trial 2: 1.64

LOQ

Trial 1: 4.78

Trial 2: 5.47

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Title:	Characterization of Organic Nitrogen Compounds and Their Impact on the Stability of Marginally Stable Diesel Fuels
Author:	Rachel D. Deese, Robert E. Morris, Alison E. Metz, et al
Publication:	Energy & Fuels
Publisher:	American Chemical Society
Date:	Jul 1, 2019

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Rachel Deese, Ph.D.

U.S. Naval Research Laboratory

Rachel Deese is a NRC postdoctoral fellow at the U.S. Naval Research Laboratory and is a member of the Chemical Sensing and Fuel Technology group. Rachel received her Bachelor's from the University of Mississippi and her Ph.D. from Louisiana State University. She has a diverse background in the realm of analytical chemistry spanning from elemental fingerprinting of Chinese drywall to performing *in situ* NMR on brine shrimp, and to now working with various analytical methods to probe fuel composition and properties.

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Alison E. Metz

Fuels Chemist

Alison is a fuels chemist for the Navy. She grew up in the small town of West Sunbury, PA and received a B.S. in chemistry from the Pennsylvania State University in 2009. After moving to Philadelphia, she earned a Ph.D. in organic chemistry from the University of Pennsylvania under the guidance of Dr. Marisa Kozlowski. While at Penn, Alison conducted research in the areas of anion-binding catalysis, cross-coupling reactions, and organic functional materials. She joined NAVAIR in 2015. During this time, she has worked with multidisciplinary teams on fuel-related research, qualification, specification updates and test method development.

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Dr. Morris is the former head of the Chemical Sensing and Fuel Technology Section at the Naval Research Laboratory. He has over 40 years of research experience involving the chemical behavior, characterization and handling of Navy mobility fuels, resulting in over 200 publications and reports, and 8 patents. Areas of his research have focused on a wide range of fundamental and mechanistic studies. Recent areas of research include development of improved sensing and modeling technologies to correlate critical fuel properties and engine performance to composition, and the development of advanced analytical tools for fuel diagnostics and prognostics. Since retiring from federal service and joining Nova Research, Dr. Morris continues to support to the Navy mobility fuels program. He is also serving as Secretary of the International Association for Stability, Handling and Use of Liquid Fuels (IASH) and is Chairman of the Advanced Research and Test Methods group for aviation fuels in the Coordinating Research Council.

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Mark Romanczyk, Ph.D.

Peraton

Mark Romanczyk graduated with a Bachelor's degree with distinction in Biology from The Pennsylvania State University and a Master's degree in Biochemistry from the University of Scranton. He graduated with a Ph.D. in Chemistry from Purdue University in 2019 working under Distinguished Professor Hilkka Kenttamaa.

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Thomas Loegel, Ph.D.

U.S. Naval Research Laboratory

Dr. Loegel received his Ph.D. in Chemistry from Miami University in 2012 before joining the Fuels Team at the US Naval Research Laboratory in Washington D.C. Under the tutelage of Dr. Robert Morris, Dr. Loegel has developed numerous instrumental methods to determine and quantify fuel additives, uncover fuel contamination, characterize fuel composition, or investigate changes in fuel chemistry.

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