Compound-specific stable carbon isotope ratios of phenols and nitrophenols derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide

Satoshi Irei [a,b,*], Jochen Rudolph [a], Lin Huang [b]

[a] Centre for Atmospheric Chemistry, Department of Chemistry, York University, 4700 Keele Street, Toronto, Ontario M3J 1P3, Canada
[b] Climate Research Division, Atmospheric Science and Technology Directorate, Science and Technology Branch, Environment Canada, 4905 Dufferin Street, Toronto, Ontario M3H 5T4, Canada

HIGHLIGHTS

- For GCC–IRMS analysis, an approach avoiding impact of NO_2 on $\delta^{13}C$ was demonstrated.
- Carbon isotope fractionations during derivatizing reactions here were negligible.
- Except some labile compounds, the overall bias of the method here was −0.21‰.
- Even for the labile compounds, measurement biases ranged +1.2‰ to −1.4‰.
- Real sample analysis demonstrates usefulness of the method for fractionation study.

GRAPHICAL ABSTRACT
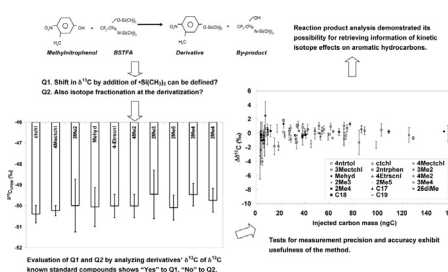
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ABSTRACT

We developed an analytical method for measuring compound-specific stable carbon isotope ratios ($\delta^{13}C$) of phenols and nitrophenols in filter samples of particulate organic matter. The method was tested on 13 phenols derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), together with four nonphenolic compounds. The data obtained by our method required two specific corrections for the determination of valid $\delta^{13}C$ values: (1) for nitro compounds, the routine correction with use of m/z 46 for the contribution of $^{12}C^{17}O^{16}O$ molecules) to m/z 45 was modified due to impact of NO_2 on the m/z 46 trace, and (2) for the derivatized phenols, measured $\delta^{13}C$ values were corrected for the shift in $\delta^{13}C$ due to the addition of carbon atoms from the BSTFA moiety. Analysis of standard-spiked filters showed that overall there was a small compound-dependent bias in the $\delta^{13}C$ values: the average bias ± the standard error of the mean of $-0.21 \pm 0.1‰$ for the standard compounds tested, except 3-methylcatechol, methylhydroquinone, 4-methyl-2-nitrophenol, and 2,6-dimethyl-4-nitrophenol, whereas the average biases ± the standard errors of the mean for those were $+1.2 \pm 0.3‰$, $+1.2 \pm 0.2‰$, $-1.2 \pm 0.2‰$, and $-1.4 \pm 0.5‰$, respectively, when the injected mass of a derivatized compound exceeded 15 ngC. In situations where such small biases and uncertainties are acceptable, the method described here could be used to obtain valuable information about $\delta^{13}C$ values. We also analyzed a real filter sample to demonstrate the practical applicability of the method.

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* Corresponding author. Current address: National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan. Tel.: +81 29 850 2696; fax: +81 29 850 2579.
E-mail address: irei.satoshi@nies.go.jp (S. Irei).

1. Introduction

Phenols and nitrophenols are ubiquitous in the atmosphere and can be found in particulate organic matter (POM), the gas phase, and rainwater [1–6]. They are formed by photooxidation of volatile organic compounds (VOCs) [7–13] and are also emitted directly from various sources [4,14]. Because these compounds are toxic and hygroscopic, they can have adverse health effects and influence the properties of cloud condensation nuclei and thereby affect radiative forcing [15]. Thus, understanding their sources, fates, reaction mechanisms, and budget in the atmosphere is important. However, because these compounds exist as complex mixtures, our current knowledge of their atmospheric sources and sinks is extremely limited.

Measurement of stable carbon isotope ratios (δ^{13}C) is a powerful tool for studying complex chemical/physical processes and reaction mechanisms because the ratios provide information that is often unavailable from concentration measurements alone. Rudolph et al. [16] reported a method for measuring compound-specific δ^{13}C values of atmospheric VOCs at sub-ppb levels with high precision by means combining GC, oxidative combustion, water removal, and high-precision isotope ratio mass spectrometry (GCC–IRMS) that was first developed by Matthew and Hayes [17]. During the last decade, many reports on the use of δ^{13}C measurements to study the atmospheric chemistry of VOCs have been published [18 and references therein]. However, only a few studies of the atmospheric photooxidation products of VOCs have been reported [6,19–21], partly because of the lack of appropriate measurement methods. Although GCC–IRMS is ideal for some compound-specific δ^{13}C measurements, its application to thermally labile atmospheric trace compounds such as nitrophenols is difficult.

Derivatization is an established means for quantitative analysis of reactive and semivolatile compounds [22]. Derivatization techniques have been used for δ^{13}C measurements by GCC–IRMS, with mass balance-based correction for the carbons in the derivatizing moiety. For example, alkyl derivatization has been used for carboxylic acids [20], dinitrophenylhydrazine derivatization for formaldehyde [21], and tert-butyldimethylsilyl derivatization for amino acids [23]. The advantages and disadvantages of using derivatization techniques for compound-specific δ^{13}C measurements have been reviewed [24]. However, to the best of our knowledge, there have been no reports of compound-specific δ^{13}C measurements for phenols, even using derivatization techniques. Silyl derivatization is often used for phenol analysis by GC (see e.g., [22]), and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide is preferred over N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) because tert-butyldimethylsilyl derivatives are more stable. However, for δ^{13}C measurements, the derivatizing reagent should contain as few carbons as possible, to reduce the magnitude of propagated uncertainties involved in correction of the measured δ^{13}C values for the carbons in the reagent.

In this paper, we present a method for measuring δ^{13}C values of phenols, nitrophenols, and nitrotoluene on filter samples by derivatization with BSTFA and subsequent GCC–IRMS analysis. 13 phenols and 4 nonphenolic compounds were used for evaluation of the method. We describe the specific corrections necessary for the method and discuss its disadvantages.

2. Experiment

2.1. GCC–IRMS measurement

Thirteen phenols and nitrophenols and four nonphenolic compounds were used as standards for developing and testing the GCC–IRMS method (Table 1). Note that some mixtures

Table 1
Standard compounds tested and their reference δ^{13}C values determined by an offline method (n = 3 or more).

Compound	Abbreviation	$\delta^{13}C_{V\text{-PDB}}$ (‰)[a]
4-Nitrotoluene	4ntrtol	−27.26 ± 0.05
4-Methylcatechol	4Mectchl	−24.44 ± 0.02
3-Methylcatechol	3Mectchl	−24.12 ± 0.00
3-Methyl-2-nitrophenol	3Me2	−27.53 ± 0.02
Methylhydroquinone	Mehyd	−26.63 ± 0.08
4-Methyl-2-nitrophenol	4Me2	−26.90 ± 0.01
2-Methyl-3-nitrophenol	2Me3	−26.66 ± 0.01
2-Methyl-5-nitrophenol	2Me5	−27.20 ± 0.02
3-Methyl-4-nitrophenol	3Me4	−25.68 ± 0.02
2-Methyl-4-nitrophenol	2Me4	−27.41 ± 0.04
Catechol	ctchl	−27.79 ± 0.10
2-Nitrophenol	2ntrphen	−27.73 ± 0.01
4-Ethylresorcinol	4-Etrscnl	−26.77 ± 0.04
2,6-Dimethyl-4-nitrophenol	26diMe	−28.99 ± 0.21
n-Heptadecane	C$_{17}$	−29.12 ± 0.03
n-Octadecane	C$_{18}$	−28.57 ± 0.04
n-Nonadecane	C$_{19}$	−35.13 ± 0.01

[a] The values shown are averages ± standard errors of the means from replicate measurements.

of these standards also contained o-cresol and 2-hydroxy-5-nitrobenzaldehyde, which were not evaluated in detail in this study. All the standards were purchased from Sigma–Aldrich (Oakville, ON, Canada) and had stated purities exceeding 99%. The δ^{13}C values of the standards were determined by conventional offline combustion followed by δ^{13}C measurement by means of an isotope ratio mass spectrometer equipped with a dual inlet (IsoPrime, GV Instruments, UK), as described elsewhere [25]. The measured δ^{13}C values are listed in Table 1.

Primary standard solutions, individual of which contain a single compound of the nitrotoluene and the phenols tested, were prepared in HPLC-grade acetonitrile (Sigma–Aldrich, Oakville, Canada). Those primary solutions were used to have different concentration mixtures of secondary or tertiary standard solutions at concentrations ranging from 2.2 to 110 μgC mL^{-1}. Owing to the limited solubility of the n-alkanes in acetonitrile, they were mixed into the phenolic solution mixtures in the following manner. First, 90 mgC mL^{-1} n-alkane standard solutions were prepared in hexane, a nonpolar solvent, and these primary solutions were used to have a mixture of secondary standard solution in hexane, then the mixture was diluted by three orders of magnitude with acetonitrile. This diluted n-alkane solution in acetonitrile was used for volumetric standards, as well as for routine evaluation of the performance of the GCC–IRMS system.

Prior to isotope ratio measurements, the phenols were derivatized with 99% pure BSTFA (Regisil, Regis Technologies). Approximately 10% (v/v) of BSTFA was added to certain amount of extracts (20–50 μL), and the mixtures were stirred for 5 min at room temperature to replace a hydrogen atom of OH group with a three-carbon trimethylsilyl (TMS) group. The δ^{13}C values of the TMS-free compounds ($\delta^{13}C_{free}$) were calculated with the following equation:

$$\delta^{13}C_{free} = \frac{\#C_{deriv}}{\#C_{free}} \times \delta^{13}C_{deriv} - \frac{\#C_{TMS}}{\#C_{free}} \times \delta^{13}C_{TMS} \qquad (1)$$

where $\delta^{13}C_{deriv}$, $\delta^{13}C_{TMS}$, $\#C_{deriv}$, $\#C_{TMS}$, and $\#C_{free}$ are the stable carbon isotope ratio of the derivatized compound, the stable carbon isotope ratio of the TMS group, the total number of carbon atoms in the derivatized molecule, the number of carbon atoms in the TMS group, and the number of carbon atoms in the TMS-free compound, respectively. We determined $\delta^{13}C_{TMS}$ by measuring the δ^{13}C values of derivatives of standard compounds with known δ^{13}C values. This approach works under the assumption that the derivatization reaction does not result in isotope fractionation.

A GCC–IRMS system consisting of a gas chromatograph (6890A, Agilent Technologies), an oxidative combustion furnace (GV Instruments), a Nafion dryer, and an isotope ratio mass spectrometer (IsoPrime, GV Instruments) was used to determine the $\delta^{13}C$ of each derivative. Note that because the instrumentation was purchased for analysis of nonnitro compounds, such as n-alkanes, it did not have a reduction furnace for converting NO_2 to N_2. Cold on-column injection was used for the analysis because this injection method was better than the split or splitless injection mode for analysis of the phenols and nitrophenol, as indicated by the results of injection tests using GC with flame ionization detection [26]. A retention gap (0.32 mm i.d. × 2 m or 0.53 mm i.d. × 1 m, Siltek fused-silica capillary, Restek Corp., USA) for one microliter injection was attached to the separation column (0.25 mm i.d. × 100 m with a 1.0-μm film of Rtx-5Sil MS, Restek Corp., USA) via a glass connector (Siltek press-tight connector, Restek Corp., USA). The other end of the separation column was attached to a Y connector (Siltek press-tight Y, Restek Corp., USA), to which a 0.32 mm i.d. × 26 cm fused-silica capillary and a 0.53 mm i.d. × 13 cm fused-silica capillary (Siltek capillaries, Restek Corp., USA) were attached. The former capillary was connected to an oxidative combustion tube (described later), and the latter was connected to a heart-split valve that guided the column effluents either to a flame ionization detector or to the oxidation tube.

The GC oven temperature was programmed as follows: ramp from 353 to 403 K at 3 K min^{-1}, isothermal hold for 5 min at 403 K, ramp from 403 to 444 K at 6 K min^{-1}, ramp from 444 to 543 K at 2 K min^{-1}, and isothermal hold for 5 min at 543 K. The inlet temperature was programmed to always be 5 K below the oven temperature. The flow rate program was as follows: 2.0 mL min^{-1} up to 53 min, then ramp to 3.1 mL min^{-1} at 10 mL min^{-1}, and then hold at 3.1 mL min^{-1} for 30 min. Under the separation conditions described above, solutions containing only a TMS derivative of a single compound were analyzed by the GCC–IRMS to confirm the retention time of the derivative.

The commercially supplied heated interface between the gas chromatograph and the oxidation furnace was removed for this analysis because the extra heat often caused decomposition of the standards. Oxidative combustion was carried out in a thermally conductive gas-tight ceramic tube (40 cm length × 0.5 mm i.d. × 6.5 mm o.d., Bolt Technical, Houston, TX, USA). Thin high-purity platinum, copper, and nickel wires (Alfa Aesor, Ward Hill, MA, USA) were braided and installed inside the tube. Prior to use, the oxidation tube was conditioned at 1323 and 773 K several times under a flow of helium containing enough pure oxygen (Praxair Canada Inc., Mississauga, ON, Canada) so that the oxygen level inside the tube was high enough to maintain a constant stable oxygen isotope ratio but low enough to not interfere with the IRMS analysis. For routine measurements the oxidation tube was used at 1223 K.

For every measurement, reference CO_2 gas (Praxair Canada Inc., Mississauga, ON, Canada) was injected via the dual inlet of the IRMS five times for 20 s each time. The $\delta^{13}C$ of the reference CO_2 was regularly calibrated using carbonate standards, as described in detail by Huang et al. [27].

2.2. Peak integration and isotope ratio determination

The chromatographic raw data for the m/z 44–46 traces were saved digitally with 0.1-s resolution. Peak areas were estimated by a summation method similar to that described by Ricci et al. [28]. Perpendicular drop integration was used here because relative change of peak area counts determined by the means of integration is less sensitive to the definition of peak boundaries due to the large peak area counts.

To determine the peak areas, we started by manually defining the peak boundaries. The criteria and procedures used to optimize the definition of the peak boundaries are described in Section 3.1. To avoid bias caused by small differences in manually defined peak boundaries, we used an average of the manually defined relative peak boundaries (relative to the width at half-height of the m/z 44 peak) for a specific compound to select peak boundaries for m/z 44–46. The start and end points of the peaks were treated separately. The algorithm was as follows, using the start point as an example: The manually chosen start point ($^i t_s^*$), the center of the peak ($^i t_c$), and the width at half-height for the front half of the peak ($^i w_{front}$) were determined for each peak of substance i. From these values, the start point relative to $^i w_{front}$ ($^i f_s^*$) was then calculated:

$$^i f_s^* = \frac{^i t_c - {}^i t_s^*}{^i w_{front}} \tag{2}$$

The $^i f_s^*$ values determined from all the chromatograms for substance i were averaged ($^i F_s$), and $^i F_s$ was then used to determine the start point ($^i t_s$) that was actually used to determine peak area:

$$^i t_s = {}^i t_c - {}^i F_s \times {}^i w_{front} \tag{3}$$

The same procedure was used to determine the end points for peak integration ($^i t_e$). The peak baseline was defined by drawing a line between two points, each of which was the average of 30 consecutive data points believed to be the background before and after the estimated peak. Finally, the signal intensities between $^i t_s$ and $^i t_e$ were summed, and peak area was then calculated by subtracting the baseline area count from the sum. We carried out this procedure for the m/z 44–46 traces by using the $^i F_s$ value obtained from the m/z 44 trace.

Because ^{17}O in CO_2 molecule also contributes to the m/z 45 trace, we applied a correction using the m/z 46 signal for the calculation of $\delta^{13}C$ [29]. However, for compounds containing nitrogen atoms, small amounts of NO_2 can form in the oxidation furnace, and this NO_2 contributes to m/z 46. To avoid bias in measured $\delta^{13}C$ values for nitro compounds, we therefore used the average $^{17}O/^{18}O$ ratio from the results of nitro-free compound analysis for the correction.

We then calculated $\delta^{13}C$ (in per mil, ‰) as follows:

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{reference}} - 1 \right] \tag{4}$$

where $(^{13}C/^{12}C)_{sample}$ and $(^{13}C/^{12}C)_{reference}$ are the $^{13}C/^{12}C$ atomic ratios of the sample and reference CO_2 gases, respectively. Note that the atomic ratios are the ^{17}O-corrected ratios of m/z 45 to m/z 44. All $\delta^{13}C$ values presented here are relative to the Vienna Pee Dee Belemnite scale.

2.3. Sample preparation

The GCC–IRMS method presented here is intended to be used for analysis of POM collected from the gas phase onto glass fiber filters coated with polytetrafluoroethylene (47 mm diameter, Pallflex Fiber Film, Pall Corp.). The overall procedure involved solvent extraction, reduction of the extract volume by evaporation, derivatization, and GC–MS and GCC–IRMS measurements. The method was first evaluated by analysis of filters spiked with variable volumes of acetonitrile solutions of standards such that the spiked masses ranged from 0.6 to 14 μgC. After being spiked, the filters were allowed to dry for ∼5 min and then extracted in 60 mL wide-mouth amber jars (Chromatographic Specialties, Inc., Brockville, ON, Canada), the inner surfaces of which were pre-etched and pre-silanized using a mixture of hydrofluoric and hydrochloric acid and dimethyldichlorosilane (Sigma–Aldrich, Oakville, ON, Canada), with 3 mL of HPLC-grade acetonitrile with ultrasonic agitation for 3–5 min. The extracts were filtered with 0.45 μm pore size PTFE
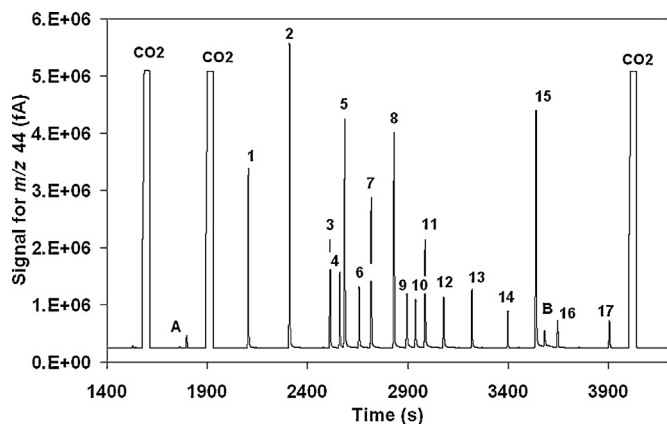
Fig. 1. GCC–IRMS chromatogram of standard compound mixture with BSTFA (injected mass 6–63 ngC). The peaks are labeled as follows: (1) catechol, (2) 4-nitrotoluene, (3) 4-methylcatechol, (4) 2-nitrophenol, (5) 3-methylcatechol, (6) 3-methyl-2-nitrophenol, (7) methylhydroquinone, (8) 4-ethylresorcinol, (9) 4-methyl-2-nitrophenol, (10) 2-methyl-3-nitrophenol, (11) 2-methyl-5-nitrophenol, (12) 3-methyl-4-nitrophenol, (13) 2-methyl-4-nitrophenol, (14) n-heptadecane, (15) 2,6-dimethyl-4-nitrophenol, (16) n-octadecane, and (17) n-nonadecane, respectively. Peaks A and B are o-cresol and 2-hydroxy-5-nitrobenzaldehyde, respectively, which were not evaluated in this study.

Fig. 2. Dependence of $\delta^{13}C$ of the 2-methyl-4-nitrophenol derivative on the end point of the peak integration relative to the peak width at half-height ($^{2Me4}f_e^*$) for injected masses of 5 ngC (open circles) and 110 ngC (solid circles). The data points are averages of three replicate measurements, and the error bars indicate SDs. The estimated peak starts and the baseline points were fixed in this evaluation, and perpendicular drop integration was used to determine peak areas.

syringe filters (Chromatographic Specialties Inc., Brockville, ON, Canada), and each filtrate was concentrated in a 5 mL conical vial (Reacti-Vial, Pierce Chemical Co., Rockford, IL, USA) under a gentle flow of nitrogen gas. The residue left in the jars after the primary extraction was rinsed three times with 2 mL of acetonitrile, and the rinse solutions were also filtered and combined with the concentrated primary extract. The volume of the combined extract was further reduced to approximately 0.1 mL under nitrogen. Defined volumes of n-alkanes mixtures in acetonitrile were added to a 20–100 μL aliquot of the concentrated extract to serve as volumetric standards for accurate determination of the final volume. The mass of n-alkanes added ranged from 1 to 8 μgC. The derivatization procedure for the extracts was the same as that for the standard solution described earlier. The extracts were derivatized and analyzed on the same day that they were extracted.
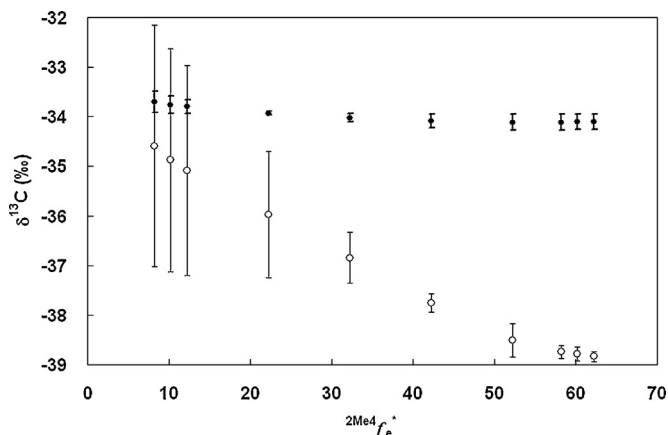
2.4. Test for applicability of method

The method developed here was tested for analysis of a real filter sample. Secondary POM was generated by gas-phase photooxidation of toluene (-26.72 ± 0.01‰) in a flow reactor [25,30] and collected on a PTFE-coated glass fiber filter (Pallflex, Fiberfilm, 47 mm disk filter, Pall Science), which has 96.4% filtration efficiency at 300 μm DOP particles. The filter was extracted, and the extract was analyzed in the same manner described earlier. The unknown compounds in the extract were identified by GC–MS (Saturn 2000, Varian Canada, Mississauga, ON, Canada) and their $\delta^{13}C_{free}$ values were determined by GCC–IRMS.

3. Results and discussion

3.1. Peak integration

An example of a chromatogram obtained by means of the GCC–IRMS method is shown in Fig. 1 (the scale of the x-axis is expanded). Note that in each chromatogram, the average ratio of m/z 45 to m/z 44 and the average ratio of m/z 46 to m/z 44 calculated for the five CO_2 reference peaks were used for determination of the $\delta^{13}C$ values of the target compounds. Most of the peaks in the chromatogram shown in the figure were baseline separated. However, there were slight overlaps between the peaks for 3-methylcatechol

and 2-nitrophenol as well as between the peaks for 2,6-dimethyl-4-nitrophenol and 2-hydroxy-5-nitrobenzaldehyde. Note that there was considerable deterioration of the shape of the 2-hydroxy-5-nitrobenzaldehyde peak and the height of the o-cresol peak, most likely because of the high reactivity of 2-hydroxy-5-nitrobenzaldehyde and the volatility of o-cresol, respectively. Therefore, we concluded that the developed method was not suitable for these two compounds, and they were not evaluated further.

Although the peak starts could be clearly defined because the peaks rose sharply from the baseline, the peak ends were sometimes difficult to define because of peak tailing and baseline drift. Consider, for example, the dependence of the $\delta^{13}C$ value for the 2-methyl-4-nitrophenol derivative as a function of the end point of the peak integration relative to the peak width at half-height ($^{2Me4}f_e^*$), which is shown in Fig. 2 for injected masses of 5 and 110 ngC. The results for the 110 ngC injections showed good reproducibility (at least 0.3‰); the differences in $\delta^{13}C$ for different values of $^if_e^*$ were statistically insignificant. In contrast, for the 5 ngC injections, the $\delta^{13}C$ depended strongly on the integration end point; the bias and the reproducibility for the 5 ngC injected mass improved significantly and systematically as the width of the $^if_e^*$ increased. The bias is likely because of the impact of the background, as has been pointed out by Brenna et al. [31]. The choice of an optimum integration window was somewhat arbitrary and depended on a trade-off between reproducibility and bias, therefore, we decided that for each individual substance i it was reasonable to use the results for the largest injected carbon mass as the reference for determination of ^iF_s and ^iF_e described in Section 2.2.

3.2. Applied corrections

As mentioned above, the m/z 45 to m/z 44 ratio was corrected for the contribution of $^{16}O^{12}C^{17}O$ molecules to the m/z 45 trace, according to the established procedure described by Allison et al. [29] with one exception: the average of the m/z 46 to m/z 44 ratios obtained for the nitro-free compounds was used for all the ^{17}O corrections to avoid bias due to NO_2 molecules. The average ratios of m/z 46 to m/z 44 ($\pm$SD) for the phenols, nitrophenols, and n-alkanes were $3.994 \times 10^{-3} \pm 0.006 \times 10^{-3}$, $4.3 \times 10^{-3} \pm 0.1 \times 10^{-3}$, and $4.002 \times 10^{-3} \pm 0.003 \times 10^{-3}$, respectively. As the average ratios of m/z 46 to m/z 44 for the phenols and nitrophenols were applied to the $\delta^{13}C$ calculation for the 2-methyl-4-nitrophenol derivative, the difference between the $\delta^{13}C$ values was ~2.5‰, whereas the
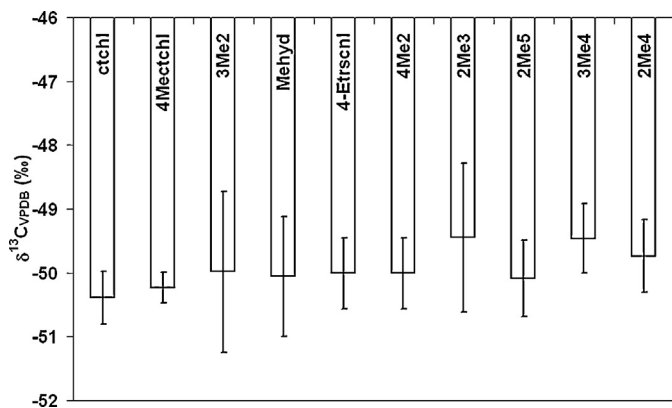
Fig. 3. Average δ^{13}C of the TMS group of BSTFA estimated on the basis of the measured δ^{13}C values of derivatives of standards ($n = 9$). The error bars represent standard errors of the mean. The results shown were obtained for injected masses between 27 and 157 ngC.

Fig. 4. Difference between the $\delta^{13}C_{free}$ values measured by GCC–IRMS and reference δ^{13}C values ($\Delta\delta^{13}$C) as a function of injected carbon mass. The data points are averages of three replicate measurements.

average ratios for the phenols and the n-alkanes applied, the difference between the values was ~0.05‰. The significant differences between the nitro-free phenols and the nitrophenols as well as the large SD of the m/z 46 to m/z 44 ratio for the nitro compounds can be explained in terms of the influence of NO_2 formed at the combustion interface. Our results indicate that in our GCC–IRMS system, NO_2 produced from the nitro compounds, which contained less than ~1% nitrogen, resulted in significant bias in the δ^{13}C values, whereas oxygen atoms in the phenols did not, as indicated by the comparison between the results for the phenols and the n-alkanes. The small SDs of the m/z 46 to m/z 44 ratios for the nitro-free compounds suggested that use of the m/z 46 to m/z 44 ratio derived from all the nitro-free compounds (i.e., the n-alkanes and the phenols) did not result in significant additional uncertainty or bias. Therefore, we used the average of the m/z 46 to m/z 44 ratios from the nitro-free compounds, $3.997 \times 10^{-3} \pm 0.008 \times 10^{-3}$, for all the ^{17}O corrections.

We determined $\delta^{13}C_{TMS}$ values by using Eq. (1), the δ^{13}C values of the standard compounds (Table 1), and the results of GCC–IRMS measurements for the derivatives of the highest concentration mixture we prepared (Fig. 3). Completion of derivatization was confirmed by linearity of quantitative calibration ($r^2 > 0.95$). The figure shows the average $\delta^{13}C_{TMS}$ with standard error of the mean for each standard derivative based on triplicate measurements of three standard solution mixtures. It should be noted that 2-nitrophenol, 3-methylcatechol, 2,6-dimethyl-4-nitrophenol, and 2-hydroxy-5-nitrobenzaldehyde were excluded for $\delta^{13}C_{TMS}$ determination due to incomplete peak separation from the neighboring peaks. The means of the $\delta^{13}C_{TMS}$ determination assumed that the derivatization reaction itself does not result in carbon isotope fractionation. The assumption was thought be reasonable because the silylation reaction did not involve the formation or breaking of any chemical bonds involving carbon atoms [24], and even derivatizing reaction involving carbon bonds did not result in significant isotope fractionation [20,21]. The figure exhibits that the $\delta^{13}C_{TMS}$ results agreed with our expectation that there was no significant bias depending on the standard compounds. Although there is no way to independently verify the accuracy of the $\delta^{13}C_{TMS}$ values, the small variability of the values between compounds indicates that the use of the average $\delta^{13}C_{TMS}$ gave consistent results. The average $\delta^{13}C_{TMS}$ (±standard error) over all the standard derivatives was -49.94 ± 0.33‰ ($n = 90$). The 0.33‰ uncertaintiy propagates to ~0.1‰ and ~0.3‰ uncertainties of $\delta^{13}C_{free}$ for methylnitrophenols and catechols, respectively. This average value and its standard error were used for evaluation of the measurement accuracy and precision.
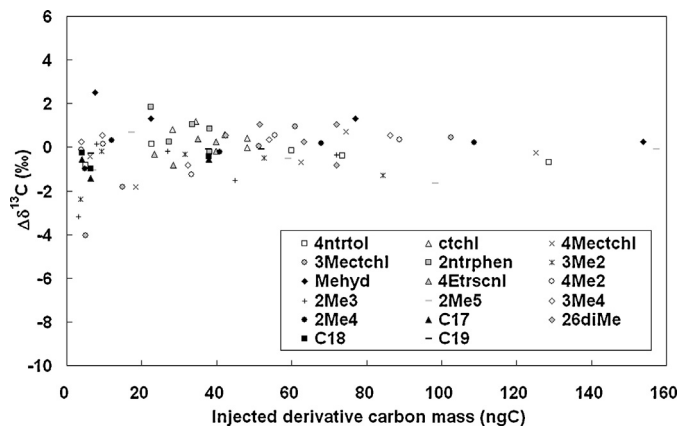
3.3. Analysis of standard solutions

We determined the difference between the reference δ^{13}C values shown in Table 1 and the $\delta^{13}C_{free}$ values determined by means of the GCC–IRMS analysis (i.e., reference δ^{13}C – measured $\delta^{13}C_{free}$; designated $\Delta\delta^{13}$C) and plotted the resulting values (averages ± SDs from replicate measurements) as a function of carbon mass injected into the GCC–IRMS system (Fig. 4). Carbon masses injected were varied by one microliter injection of difference concentration mixtures. The $\Delta\delta^{13}$C values varied randomly from -1.7‰ to $+1.8$‰ for injected masses between 20 and 158 ngC, whereas the range of variation was larger (-2‰ to $+2.5$‰) for injected masses less than 20 ngC. The measurement accuracy clearly depended on the injected carbon mass. As looked more closely, the accuracy seemed to depend on compound: the $\Delta\delta^{13}$C for 4-methylcatechol, 3-methylcatechol and methylhydroquinone exceeded ~+2‰ or -2‰ as injected carbon mass was less than 18 ngC, while for other compounds the $\Delta\delta^{13}$C exceeded -2‰ as injected mass was less than 4 ngC. During the analysis, we found that some compounds, such as 3-methylcatechol and 4-methylcatechol, were sensitive to even minor contamination in the GC system. Even though rinsing the GC column with solvent and replacing the retention gap and the liner of the injector improved the accuracy, peak deterioration (i.e., smaller peak heights, substantially asymmetric peak shapes, appearance of peak shoulders, etc.) with small injected masses (<18 ngC) added additional uncertainty to the measured δ^{13}C values for the methylcatechols. In overall, we conclude that excepting the catechols, the average $\Delta\delta^{13}$C was 0.03‰ with a SD of 0.72‰ and a standard error of 0.1‰ for injected masses greater than 4 ngC.($n = 71$). For the catechols, biases seem to appear as injected carbon masses were less than 18 ngC.

3.4. Filter blank and standard spike tests

When a blank filter was analyzed, peaks for two unknown contaminants appeared near the peaks for 4-ethylresorcinol and 2,6-dimethyl-4-nitrophenol. However, these peaks did not significantly overlap with the peaks of the standards.

The average recovery yields for four standard spike tests for each compound ranged from 82% to 107%, and the SDs ranged from 2% to 14% for spiked masses between 0.6 and 11 μgC (Table 2). All the compounds except 2-nitrophenol and 4-ethylresorcinol showed recovery yields of 100% within the SDs. The recovery yields of 2-nitrophenol and 4-ethylresorcinol were significantly lower (82 ± 8%) and higher (107 ± 2%) than 100%, respectively. This is perhaps because of variations in the effect of the baseline. The

Table 2
Results of standard spike tests ($n = 4$).[a]

Substance[b]	Spiked carbon mass (μgC)	Average recovery yield (%)	SD	Average $\Delta\delta^{13}C$ (‰)	SD	1σ[c]
4ntrtol	2.3–10.7	107	5	0.04	0.23	0.11
ctchl	1.6–2.0	96	11	0.25	1.54	0.77
4Mectchl	3.7–5.6	94	8	0.11	0.59	0.3
3Mectchl	3.1–7.4	84	14	1.2	0.6	0.3
2ntrphen	1.7–2.2	82	8	0.61	0.34	0.17
3Me2	0.7–4.9	99	2	0.11	0.25	0.12
Mehyd	1.2–6.9	98	7	1.2	0.4	0.2
4-Etrscnl	1.5–2.0	107	2	0.09	0.37	0.19
4Me2	0.7–5.2	90	5	−1.2	0.4	0.2
2Me3	0.6–4.2	101	3	−0.75	0.32	0.16
2Me5	1.2–9.2	99	5	−0.33	0.39	0.19
3Me4	0.7–5.0	99	7	−0.7	0.5	0.25
2Me4	0.8–6.3	103	8	−0.45	0.6	0.3
26diMe	3.5–4.6	99	7	−1.4	0.9	0.5
C_{17}	n.a.[d]	n.a.[d]	n.a.[d]	−0.74	0.4	0.2
C_{18}	n.a.[d]	n.a.[d]	n.a.[d]	−0.72	0.16	0.08
C_{19}	n.a.[d]	n.a.[d]	n.a.[d]	−0.21	0.17	0.08

[a] Values shown in the table are mean values of the results from the four extraction tests.
[b] See Table 1 for the abbreviations.
[c] Standard error of the mean (i.e., SD divided by the square root of four).
[d] Not applicable.

results were still acceptable because the yields were close to 100%, and no correlation between the yields and the measured $\delta^{13}C$ was observed. The overall average recovery yield was 97% with a SD of 7% and a standard error of 2%.

The $\Delta\delta^{13}C$ values (i.e., measurement biases) varied from −1.4‰ to +1.2‰ (Table 2). Unlike the results of the standard solution analysis previously discussed, the results for 4 of the 17 standard compounds (i.e., 3-methylcatechol, methylhydroquinone, 4-methyl-2-nitrophenol, and 2,6-dimethyl-4-nitrophenol) appear to show the $\Delta\delta^{13}C$ values larger than the 99% confidence level (CL) estimated by three times of the standard errors of the mean shown in Table 2, indicating statistically significant biases. Although the biases were not dependent on injected masses that were between 15 and 290 ngC as derivative carbon, those seemed to be compound dependent. Analysis of variance was performed to statistically evaluate overall $\Delta\delta^{13}C$ by this method. One-sided F test gave the F value of 6.5 for the $\Delta\delta^{13}C$ data set of 17 standard compounds over the four standard spike tests. This value was larger than 2.4, the upper critical value of F at 1% CL for a data set having the same degrees of freedom as those for the $\Delta\delta^{13}C$ data. The larger F value than the upper critical F value means that the average $\Delta\delta^{13}C$ over the 17 compounds does not represent the average over all the compounds. However, the F value for $\Delta\delta^{13}C$ data excluding the 4 compounds referred above resulted in 2.5, which is smaller than 2.7, the upper critical value of F at 1% CL for a data having the same degrees of freedom. This means that the average $\Delta\delta^{13}C \pm$ the standard error of the mean, which is −0.21 ± 0.1‰, can represent the overall bias for the standard compounds selected. In contrast, the biases ± standard errors of the mean for 3-methylcatechol, methylhydroquinone, 4-methyl-2-nitrophenol, and 2,6-dimethyl-4-nitrophenol were +1.2 ± 0.3‰, +1.2 ± 0.2‰, −1.2 ± 0.2‰, and −1.4 ± 0.5‰, respectively, with the injected carbon masses larger than 15 ngC. As discussed in the section 3.3, there is no significant mass-dependent bias for the methylnitrophenols and the catechols with the injected carbon masses larger than 4 ngC and 18 ngC, respectively. Compared to those results, the biases for the catechols here seemed to be compatible with the observed mass dependent biases, while the biases for the methylnitrophenols here did not. We suspect that unfavorable reactions causing small but significant isotope fractionations took place for the methylnitrophenols during the GCC–IRMS measurements (i.e., at the retention gap, the separation column, the

capillaries after the separation column, or all three) or during filter extraction and solvent evaporation. Another possibility is that the influence of baseline became increasingly important as the number of injections increased, possibly as a result of cross contamination from the products of decomposition of the derivatives in the GC system. Comparison of the uncertainties for the biases stated above (±0.1‰ to ±0.5‰) with the expected uncertainty propagated from the $\delta^{13}C_{TMS}$ (±0.1‰ to ±0.3‰) reveals that the magnitude of the uncertainties are comparable: the major source of the uncertainties is the uncertainty of $\delta^{13}C_{TMS}$.

In summary, we observed a small bias of −0.21 ± 0.1‰ on average through the overall procedure for the compounds except 3-methylcatechol, methylhydroquinone, 4-methyl-2-nitrophenol, and 2,6-dimethyl-4-nitrophenol. These four compounds showed the larger biases (±standard errors of the mean) ranging from −1.4 ± 0.5‰ to 1.2 ± 0.3‰.

3.5. Analysis of POM filter sample

Extracts prepared from the secondary POM filter samples were analyzed by the GC–MS for identification of compounds (Fig. 5). The results from the mass spectrometric analysis indicated the presence of the following seven compounds: 4-nitrophenol, 3-methyl-4-nitrophenol, 2-methyl-4-nitrophenol, three methyl-nitrocatechol isomers, and a nitrocatechol isomer. Although the structural isomers could not be further identified, their $\delta^{13}C_{free}$ values could nevertheless be determined by GCC–IRMS analysis. As an example, results of GCC-IRMS analysis for three secondary POM extracts are shown in Table 3. The peak areas for many of the identified products were within the range of the peak areas tested, but some of catechols were under 18 ngC injection, meaning that there may be biases on their $\delta^{13}C_{free}$ by 2‰ or higher. 4-Nitrophenol has had the highest $\delta^{13}C_{free}$ (−26.68 ± 0.61‰), and 3-methyl-4-nitrophenol the lowest (−36.5 ± 1.8‰). The $\delta^{13}C_{free}$ values of other products fell between −32‰ and −33‰. In many cases, the differences between the measured $\delta^{13}C$ values were significantly different from the initial $\delta^{13}C$ of toluene (∼27‰) as well as significantly larger than the uncertainties of the measurements. The measured $\delta^{13}C$ values seem to indicate reasonable kinetic isotope effects when compared with the $\delta^{13}C$ for the total POM carbon produced by the photooxidation of toluene [25,32].

Table 3
$\delta^{13}C_{free}$ values for toluene-oxidation products found in extract of secondary POM filter samples.[a]

Product identified	Sample A extract 17% toluene reaction		Sample B extract 27% toluene reaction		Sample C extract 10% toluene reaction	
	$\delta^{13}C$ (‰)	Concentration (ngC µL^{-1})	$\delta^{13}C$ (‰)	Concentration (ngC µL^{-1})	$\delta^{13}C$ (‰)	Concentration (ngC µL^{-1})
4-Nitrophenol	-26.68 ± 0.61	11.8 ± 0.3	-27.8 ± 1.3	33.3 ± 0.9	-26.43 ± 0.33	8 ± 2
3-Methyl-4-nitrophenol	-36.5 ± 1.8	3.1 ± 0.1	-33.98 ± 0.21	16.1 ± 0.4	n.d.	n.d.
2-Methyl-4-nitrophenol[b]	-32.57 ± 0.25	257 ± 1	-30.50 ± 0.27	524 ± 4	-33.07 ± 0.70	110 ± 20
Methylnitrocatechol isomer 1	-32.15 ± 0.26	14.4 ± 0.4	-31.61 ± 0.56	30.1 ± 0.8	-32.85 ± 0.01	7.54 ± 0.08
Methylnitrocatechol isomer 2	-33.72 ± 0.64	7.2 ± 0.2	-32.41 ± 0.55	15.9 ± 0.4	n.d.	n.d.
Methylnitrocatechol isomer 3[b]	-32.52 ± 0.52	58 ± 1	-33.98	130 ± 4	-32.73 ± 0.52	31.2 ± 0.6
Nitrocatechol isomer	-32.11 ± 0.30	7.5 ± 0.2	-32.12 ± 0.57	13.9 ± 0.2	-34.95 ± 1.05	11.1 ± 0.3

[a] Means ± SDs from triplicate measurements.
[b] Diluted samples were analyzed due to high concentration.
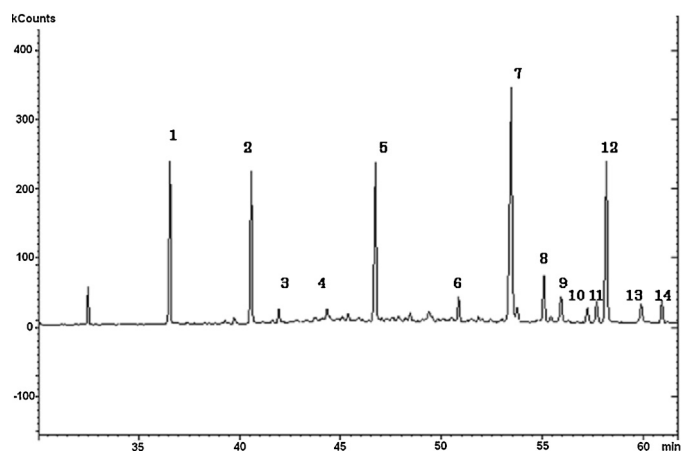
Fig. 5. Total ion chromatogram of secondary POM extract obtained by GC–MS analysis. The numbered peaks from 1 to 14 correspond to 2-nitrophenol (IS), 4-ethylresorcinol (IS), 4-nitrophenol, 3-methyl-4-nitrophenol, 2-methyl-4-nitrophenol, heptadecane (IS), 2,6-dimethyhl-4-nitrophenol (IS), methylnitrocatechol isomer 1, octadecane (IS), unknown, methylnitrocatechol isomer 2, methylnitrocatechol isomer 3, nitrocatechol isomer, and nonadecane (IS), respectively. "IS" in brackets stands for an internal standard.

4. Conclusions

Our results demonstrate that our newly developed GCC–IRMS method was useful for compound-specific $\delta^{13}C$ measurement for phenols and nitrophenols derivatized with BSTFA. Derivatization with BSTFA eliminated problems resulting from the limited thermal and chemical stability of the phenols. However, determination of the $\delta^{13}C$ values of the underivatized compounds required determination of the $\delta^{13}C$ of the added TMS group, which in turn required the availability of standard compounds with known $\delta^{13}C$ values. Our GCC–IRMS measurements for the 10 standard compounds shown in Fig. 3 allowed us to use −49.94‰ with a standard error of 0.33‰ as $\delta^{13}C$ value for the TMS group .

The results of standard solution analysis demonstrated that for compounds except the 4-methyhlcatechol, 3-methylcatechol, and methylhydroquinone the average bias ± standard error was 0.03 ± 0.1‰ as injected carbon mass was higher than 4 ngC. For the catechols such accuracy was achieved as injected carbon mass was higher than 18 ngC. The results of standard spike tests demonstrated that recovery yields were excellent (>82%) and were often quantitative within the uncertainty of the measurements. The results also demonstrated that small overall compound-dependent biases: the average bias ± the standard error of the mean of −0.21 ± 0.1‰ for the standard compounds tested, except 3-methylcatechol, methylhydroquinone, 4-methyl-2-nitrophenol, and 2,6-dimethyl-4-nitrophenol, whereas the average biases ± the

standard errors of the mean for those were +1.2 ± 0.3‰, +1.2 ± 0.2‰, −1.2 ± 0.2‰, and −1.4 ± 0.5‰, respectively. The comparison of these uncertainties with the uncertainty of $\delta^{13}C_{TMS}$ reveals the major uncertainty source is $\delta^{13}C_{TMS}$, and additional bias source(s) was likely added during the sample preparation procedure. Analysis with the small biases with the level of the uncertainties is still acceptable, and the method here can be used to determine stable carbon isotope ratios.

When we analyzed a POM sample obtained from photooxidation of toluene in a flow reactor, the differences between the $\delta^{13}C$ values of the photochemical products and their parent compound, toluene, far exceeded the uncertainty of the measurements. Although it is beyond the scope of this paper to discuss the origin of these differences, our results indicate that the developed method is a promising tool for gaining $\delta^{13}C$ of phenols and nitrophenols.

Acknowledgements

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