Quantitative carbon detector for enhanced detection of molecules in foods, pharmaceuticals, cosmetics, flavors, and fuels†

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Analysis of trace compounds, such as pesticides and other contaminants, within consumer products, fuels, and the environment requires quantification of increasingly complex mixtures of difficult-to-quantify compounds. Many compounds of interest are non-volatile and exhibit poor response in current gas chromatography and flame ionization systems. Here we show the reaction of trimethylsilylated chemical analytes to methane using a quantitative carbon detector (QCD; the Polyarc™ reactor) within a gas chromatograph (GC), thereby enabling enhanced detection (up to 10x) of highly functionalized compounds including carbohydrates, acids, drugs, flavorants, and pesticides. Analysis of a complex mixture of compounds shows that the GC-QCD method exhibits faster and more accurate analysis of complex mixtures commonly encountered in everyday products and the environment.

Detection and accurate quantification of complex organic compounds within foods, fuels, consumer products, and the environment remains a significant technical challenge for the 21st century. Humans, animals, and the environment are regularly exposed to mixtures comprising hundreds or thousands of chemicals containing potentially hazardous compounds such as pesticides and contaminants. For example, fuels derived from petroleum and biomass processing involve complex liquid mixtures (10^2–10^4 compounds) which have been found in soils and marine ecosystems. Chemicals within food and materials including Bisphenol A (BPA), phthalates, parabens, and pesticides (e.g., glyphosate) have been identified as undesirable components with potential implications for human health. Contaminants within wastewater include pharmaceuticals such as estrogen. Recently, a common ingredient called benzophenone-2 (BP-2) in soaps, cosmetics, fragrances, and sunscreen has been identified as toxic to ocean coral. Presence of trace amounts of these chemicals within complex mixtures combined with the variable detection sensitivity based on their chemical functionality leads to the difficult and sometimes impossible rigorous quantification with modern analytical methods.

A common feature of many organic chemicals of consumer interest is their significant heteroatom composition (i.e., the presence of O, N, etc.), which reduces volatility and diminishes chemical detection sensitivity in the flame ionization detector (FID). The low volatility of these compounds necessitates the use of low resolution condensed-phase methods such as liquid chromatography or derivatization methods to improve volatility for higher sensitivity gas chromatography (GC) methods. Furthermore, the high heteroatom content of common molecules such as xylose, ascorbic acid (vitamin C), or 2,5-furandicarboxylic acid (FDCA) results in low and variable response in common chemical detectors such as the FID. Thus, these types of important chemicals are generally more difficult to identify and quantify in consumer products, human tissue, and environmental samples.

A solution to enhancing the detection of highly functionalized organic molecules utilizes trimethylsilylation of heteroatom functional groups. Functional groups such as hydroxys (–OH) are reacted with a ‘silylating agent’ to form new trimethylsilyl functional groups which enhance the volatility of the molecule. The silylation of heavy molecules (Fig. 1) transforms them into higher volatility analogues of the starting molecules for use in high resolution gas separation methods (e.g., gas chromatography), which can separate hundreds of chemical compounds in a single injection. However, the detectors used in gas chromatography, such as the thermal conductivity detector (TCD) or FID exhibit low and variable response to heavily functionalized molecules, whether or not they undergo trimethylsilylation, and thus require higher concentrations and cumbersome calibration of detector response to accurately quantify chemical compounds. Improved detector sensitivity and uniform detector response would enable higher quantification accuracy, lower detection limits, time savings,
and the elimination of errors associated with the preparation of calibration standards.

Here, we evaluate the quantification of trimethylsilylated molecules representative of various industries and products using a Polyarc™ reactor for quantitative carbon detection (QCD) placed immediately before the FID (henceforth referred to as the GC/QCD-FID). The reactor is a multi-chamber catalytic microreactor depicted in Fig. 2A designed to integrate within gas chromatographs between the column and the FID and convert all molecular carbon to methane. Individual analytes eluting from the column undergo complete combustion (conversion, $X_C > 99.9\%$) followed by complete reduction to methane ($X_{CO_2} > 99.9\%$); reactions occur within catalytic micro-channels (depicted in Fig. 2B) engineered to eliminate axial dispersion and back-mixing to maintain chromatographic resolution. The resulting response of the FID to the reacted molecules is equivalent on a per carbon basis, because only methane is ionized in the flame; thus, the calibration of individual compounds becomes unnecessary.20

Examined organic compounds were selected to represent various industries including: (a) food (glucose, xylose, cellobiose), (b) polymers (terephthalic acid, furandicarboxylic acid), (c) pharmaceuticals (acetaminophen, isobutylphenylpropanoic acid or ibuprofen), (d) flavoring (vanillin, cinnamic acid), (e) vitamins (ascorbic acid or vitamin C), (f) consumer products (butylparaben, nonylphenol), and (g) pesticides (dicamba). Silylation of these compounds led to the replacement of H atoms of hydroxyl groups with trimethylsilyl (TMS) moieties as depicted in Fig. 1 (details in the ESI†) by reaction with one of three different silylating agents: (i) N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), (ii) trimethylchlorosilane (TMCS), or (iii) 1-(trimethylsilyl)imidazole (TMSI). To silylate a compound, approximately 10 ± 3 mg of the compound was added to a 1.5 mL amber screw top vial. Most compounds were silylated using BSTFA + 1% TMCS, but TMSI was used for glucose, xylose, and cellobiose. Pyridine (750 µL) was selected as a polar solvent to drive the reaction to completion and was combined with 750 µL of silylating agent. Solvents were added to the resulting mixtures to provide quantitative internal standards; these included heptane, tetrahydrofuran (THF), and ethyl acetate depending on their chemical compatibility with the analytes. Samples were then shaken for one minute to aid in mixing, and the vial was sealed with paraffin film to avoid contamination. The vials were then sealed in polyethylene bags and placed in a water thermal bath at 60 °C for one hour (terephthalic acid was heated an additional three hours to ensure complete substitution). After heating, the compound was allowed to cool to room temperature and was immediately injected into the GC/QCD-FID. The extent of H atom substitution with TMS groups in each molecule is described in the ESI†. In general, every hydroxyl group of the considered analytes was replaced with a TMS group, which was verified by the absence of extra peaks in the GC chromatograms. The exceptions are noted in the ESI†.

![Fig. 1 Preparation of oxidized organic molecules via trimethylsilylation (TMS). [1],[2] furandicarboxylic acid (FDCA); [3],[4] acetaminophen; [5],[6] vanillin; [7],[8] glucose.](image1)

![Fig. 2 Polyarc™ Quantitative Carbon Detector (QCD). (A) Image of the Polyarc™ microreactor; (B) gas flows, catalytic chemistries, and heating within the Polyarc™ reactor show how carbon-containing analytes eluting from the gas chromatograph column are fully converted to methane via a two-step catalytic process.](image2)
While the GC/QCD-FID has been shown to exhibit identical response for each compound containing only carbon, hydrogen, and oxygen, the performance of the tandem catalytic system with compounds containing silicon has not been evaluated. Catalytic combustion of silylated compounds at 450 °C in the Polyarc™ QCD will result in the formation of silica (SiO₂). The injection of multiple trimethylsilylated compounds could result in the accumulation of silica that coats the combustion catalyst or occludes the dead-volume in the reactor, thereby suppressing complete oxidation and disrupting reactor performance. It is also possible to form silicon carbides, which could accumulate both solid silicon and carbon thus resulting in reduction in signal response for each organic compound. A reduction in the catalytic performance of the reactor will eventually result in a loss in FID signal and a detector response that is no longer uniform with carbon number, since full conversion to methane is not possible.

The performance of the GC/QCD-FID using the Polyarc™ reactor (Activated Research Company) was evaluated with 12 silylated compounds (Fig. 1 and Table 1); the experimental setup is depicted in Fig. 3. The Polyarc™ reactor (Activated Research Company P/N PA-SYT-A03) with an operating temperature of 450 °C was connected directly to the effluent of a capillary column (Agilent DB-5) which was connected to an Agilent 7890A split/splitless injector operating under splitless conditions. The reactor outlet was connected directly to the FID. The reactor was supported within a custom metal housing on top of the oven, and the heater/RTD assembly (Activated Research Company P/N PA-HTA-C12) was controlled with the auxiliary heater PID controller on the GC. Air and hydrogen were provided to the combustion and reduction zones, respectively, of the reactor (Fig. 3) via diaphragm-regulated flow controllers (VICI Condyne Flow Controller model FC10AS1K-AVR).

Air and hydrogen flow rates were selected using thermodynamic predictions to ensure complete combustion followed by complete methanation. The ternary diagram of Fig. 4A identifies the region (highlighted in grey) for a mixture of hydrogen (H), oxygen (O), and carbon (C) that fulfills two criteria: (i) the red line identifies the transition between complete stoichiometric combustion and incomplete combustion, and (ii) curved grey lines at temperatures of 200, 300, 400 and 500 °C form the lower bounds where >99.9% of carbon is converted to methane at equilibrium. Fig. 4B provides a zoomed-in view of the operating region for the 12 considered compounds. At the selected temperature (450 °C) and gas flow rates (33 std. cm³ min⁻¹ H₂ and 2.5 std. cm³ min⁻¹ air), all 12 compounds reside within the region of complete (>99.9%) conversion of carbon to methane at equilibrium.

Samples were transferred from the sample vial to the GC/QCD-FID using an autosampler with a 0.5 μL syringe to inject 0.02 μL of liquid. Each compound was injected in at least three trials for two experimental arrangements (with and without the QCD), and the response factors (RF) were determined for each compound using eqn (1),

\[
RF(\text{mol % C}) = \frac{\text{area}_1/\text{mol C}_1}{\text{area}_2/\text{mol C}_2}
\]

where \(\text{area}_1\) and \(\text{area}_2\) are the integrated detector response to the compound and an internal standard, respectively, and \(\text{mol C}_1\) and \(\text{mol C}_2\) are the amount of sample and internal standard injected.

As shown in Table 1, response factors were determined for each considered analyte with and without the QCD. All trimethylsilylated species were observed to pass through the gas chromatograph without decomposing. Comparison of the RF of the 12 analytes in Fig. 5 indicates that the analytes exhibited widely varying RF values by flame ionization detection which differ by almost an order of magnitude. In comparison, all trimethylsilylated species quantified by GC/QCD-FID exhibited a response factor nearly equal to one within experimental error.

When comparing the FID response with the GC/QCD-FID to GC-FID, the majority of the compounds have improved response with the introduction of the Polyarc™ reactor. Many of the compounds have a low response (RF < 0.2) with GC/FID, which can be caused by the oxygen and nitrogen heteroatoms.

### Table 1 Response factors (RF) of trimethylsilylated compounds by flame ionization detection (FID) and quantitative carbon detection (QCD) with the Polyarc™ reactor microreactor

<table>
<thead>
<tr>
<th>Trimethylsilylated Compounds</th>
<th>Response factor (RF)</th>
<th>FID</th>
<th>Polyarc-FID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.09 ± 0.10</td>
<td>1.03 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td>0.87 ± 0.16</td>
<td>0.91 ± 0.08</td>
<td></td>
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<tr>
<td>Cellobiose</td>
<td>0.29 ± 0.08</td>
<td>1.04 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>Furandicarboxylic acid (FDCA)</td>
<td>0.78 ± 0.03</td>
<td>0.98 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Terephthalic acid (TPA)</td>
<td>0.86 ± 0.14</td>
<td>0.95 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>0.74 ± 0.12</td>
<td>1.12 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen [isobutylphenylpropanoic acid]</td>
<td>0.30 ± 0.03</td>
<td>1.06 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.40 ± 0.03</td>
<td>1.10 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>0.23 ± 0.08</td>
<td>1.04 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.45 ± 0.04</td>
<td>0.94 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Butylparaben</td>
<td>0.19 ± 0.08</td>
<td>1.08 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>0.21 ± 0.08</td>
<td>1.02 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Dicamba</td>
<td>0.60 ± 0.01</td>
<td>1.03 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

*Fig. 3 The Polyarc™ QCD integrates directly with conventional gas chromatography (GC) and flame ionization detection (FID), including PID temperature control and supplemental gas flows to the catalytic chambers.*
With the Polyarc™, $R_f \sim 1.0$ for all compounds. With current analytical techniques, the assumption of a response factor of unity cannot be made, because the response factor of any component ($RF_i$) depends on the atomic structure of the specific compound being tested. However, because all molecules are converted to methane, this assumption can be made with the Polyarc™ reactor allowing for analysis of mixtures without the need to prepare calibrations.

To test the application of the silylation and GC/QCD-FID method for complex mixtures such as those encountered in the environment, food, and pharmaceuticals, a complex mixture was prepared and tested. Vanillin, ibuprofen, dicamba, nonylphenol, and ascorbic acid were prepared in a single mixture, spanning an order of magnitude in concentration. The mixture was silylated and analyzed with both GC/QCD-FID and GC-FID. Comparison of the chromatograms shown in Fig. 6A shows a negligible decrease in peak resolution and an overall increase of signal of the GC/QCD-FID compared with conventional GC-FID. Response factors for each of the analytes obtained by GC/QCD-FID were unity within experimental error as shown in Fig. 6B.

Samples of trimethylsilylated analytes containing silicon were injected over the course of four continuous months (approximately 125 injections) without noticeable variation in response factors. The cumulative amount of silicon injected into the reactor is estimated to be 0.29 mg, which upon oxidation would convert to 0.24 mm$^3$ of silica. The presence of silica showed no detrimental effect in reactor performance. It is estimated that 50 000 injections would be required to completely fill the reactor void volume with silica.

The combination of trimethylsilylation with the Polyarc™ QCD microreactor enables enhanced detection of highly oxidized analytes. For molecules such as cellobiose, ibuprofen, or butylparaben, the detector response was enhanced by an order of magnitude and an overall increase of signal of the GC/QCD-FID compared with conventional GC-FID. Response factors for each of the analytes obtained by GC/QCD-FID were unity within experimental error as shown in Fig. 6B.

Fig. 4 Thermodynamic regimes of operable QCD parameters for trimethylsilylated compounds. (A) Temperature dependence of thermodynamic feasibility for $C:\, H:\, O$ ratios to achieve 99.9% conversion to methane. The shaded region envelops stoichiometric (i.e. combustion) and thermodynamic bounds defining a region of QCD operability. (B) All compounds fall within the operable region and are converted to 99.9% methane at reaction conditions.

Fig. 5 Conventional FID (red) and Polyarc™ QCD (blue) response factors of 14 silylated compounds. Response factors are defined as shown in eqn (1).
of magnitude making it possible to work with highly diluted samples. Analysis of these compounds was completed without the need for detector calibration, thus eliminating the need for analysis of standards which saves time and reduces the potential for measurement error. Quantification of a complex mixture of analytes using the Polyarc™ QCD microreactor shows enhanced capability for analysis of compounds commonly encountered in consumer products, energy, and the environment.

Disclosure
Professor Paul Dauenhauer is a strategic adviser for Activated Research Company.

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References


