Analytical Methods

DOI: 10.1002/anie.200805545

A Quantitative Universal Detection System for Organic Compounds in Gas Chromatography with Isotopically Enriched $^{13}CO_2$**

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The search for a truly quantitative standardless and universal chromatographic detector has been a long story of success and failure.[1] The classic example is the flame ionization detector (FID) in gas chromatography: This universal detector for organic compounds still requires specific calibration, as its response for carbon is not truly compound-independent.[2] A recent success was the application of inductively coupled plasma (ICP) mass spectrometry (MS). ICPMS has been demonstrated to possess some crucial characteristics of a quantitative universal detector. In particular, the ionization degree for a given element can be made compound-independent, in both liquid[3,4] and gas chromatography,[5] to provide a generic quantitative approach without the need to use analytical standards for each compound.[3,5] This capability has been boosted by the postcolumn use of enriched stable isotopes to quantify unknown compounds or compounds for which standards were not available.[6] The only requisite to be fulfilled is isotopic equilibration between the different compounds containing the element under study that are eluted from the column and the continuously added isotopically labeled element. Postcolumn isotope dilution analysis has mostly been applied to ICP-detectable elements (trace-metal speciation).[7] However, there are only two reports so far of the use of ICPMS and the postcolumn (HPLC) addition of ^{13}C-labeled species (benzoic acid[8] and methanol[9]) for the quantification of organic compounds. Clearly, the extremely low ionization yield of carbon in the plasma and the high carbon background under normal ICP operating conditions (atmospheric pressure) seriously hamper the use of this ionization source for the detection of carbon-isotope ratios. Moreover, the main problem of ICPMS in particular and most universal quantitative detectors in general is that structural information is lost during the detection process.

GC–MS with electron ionization (EI) has become one of the most widely used techniques in laboratories all over the world. The coupling of a gas chromatograph with a mass spectrometer detector allows both structural determination and the quantification of any volatile or semivolatile compound (directly or after chemical derivatization) in a wide variety of samples. Of course, as EI is a molecular-ion source, its response is structure-specific for each single molecule to be analyzed.[10] Therefore, analytical standards for every analyte are needed for time-consuming external calibrations or standard additions if quantification is sought. Isotopically labeled compounds have also been widely used as ideal specific internal standards for very accurate quantitative GC–MS analysis.[11] Again, as a labeled standard for each analyte in a mixture should be used, this approach can be very expensive when many analytes need to be quantified. The rapid development of the pharmaceutical and pesticide industries, among others, has led to an exponential growth in the number organic compounds subjected to analysis by GC–MS and for which certified analytical standards are not always available. In this sense, the development of a truly universal and accurate quantitative detection technique with which the concentration of many chemical compounds can be determined without the need for specific analytical standards and time-consuming calibration procedures is still required.[1] This universal quantitative detection technique should also be able to provide structural information on the compounds eluted from the chromatographic column.

Herein, we present the use of postcolumn carbon-isotope dilution in combination with GC–combustion–EIMS. This patented innovative instrumental approach provides the first generic quantitative approach for every organic compound without the need for specific standards. The combustion reaction ensures quantitative conversion of every organic compound eluted from the GC column into CO_2 to provide the required isotopic equilibrium between these compounds and the isotopically enriched carbon tracer ($^{13}CO_2$) that is continuously added, prior to their combined exposure to the ionization source. Thus, compound-independent ionization is possible whatever ionization source is used. We selected EI for our experiments so that ionization could be carried out under a high vacuum to enable much higher sensitivity and lower carbon contamination. Additionally, this ionization source can provide structural information when the combustion oven is bypassed. The proof of concept and potential of this universal method for the quantification of organic compounds is demonstrated herein.

The isotopically labeled C species selected was CO_2 enriched in ^{13}C. It was prepared from Na_2CO_3 (^{13}C, 99%) and placed in a 5 L gas container pressurized to 6 atm with He. The output of the container was connected to a mass-flow controller that exactly regulated the flow of the $^{13}CO_2$ added

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[**] Financial support was provided by the FICYT (PC06-016) and technical support by KONIK-TECH. J.R.E. acknowledges the MEC (European Social Fund) for a Ramón y Cajal contract, and S.C.D. acknowledges the FICYT for a PhD grant.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200805545.

postcolumn. This mass-flow controller was connected to a "Y" piece to enable the accurate and continuous mixing of the $^{13}CO_2$ tracer with the CO_2 formed after the combustion of every organic compound originally present in the GC-column eluent (Figure 1).

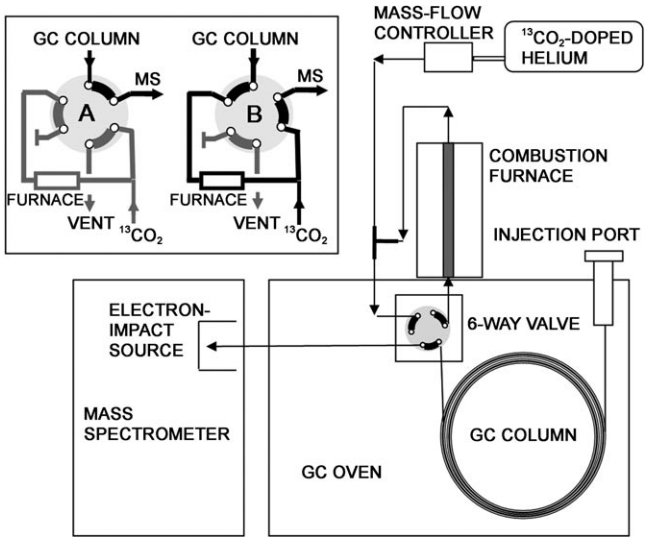

Figure 1. Schematic illustration of the setup showing the modifications made in the GC–MS instrument. The two possible valve configurations are shown at the top left: A, for structural identification by conventional GC–MS analysis, and B, for absolute quantification by postcolumn isotope dilution.

The home-made combustion furnace consisted of a ceramic tube (internal diameter: 0.5 mm) in which copper wires, previously oxidized to CuO, were introduced and maintained at a suitable temperature (> 800 °C).[12] Platinum wires were also introduced to catalyze the combustion reaction. Figure 1 shows the whole instrumental setup. Minimal modification of a standard GC–MS system is required. Since we selected $^{13}CO_2$ as the tracer, efficient gaseous mixing is possible subsequent to the combustion reaction. The introduction of a six-way valve made it possible to operate the system in two configurations (A and B at the top left of Figure 1). In configuration A, the column is connected directly to the mass spectrometer, as in any commercial GC–MS instrument, to provide structural information on every organic compound present in the sample. If the valve is switched to configuration B, subsequent injection provides absolute quantitative data for each previously identified compound by using the postcolumn isotope dilution approach. Such a valve also prevents the solvent from entering the combustion furnace if the valve is kept in configuration A at the beginning of the analysis and only switched to configuration B when the solvent has already eluted. The same time delay is applied to the switching on of the electron-impact filament.

The performance of the setup was initially tested with a single organic compound, tetradecane. Figure 2 shows the chromatogram obtained at *m*/*z* 44 and 45, which correspond to $^{12}CO_2^+$ and $^{13}CO_2^+$, respectively. The measured ratio of

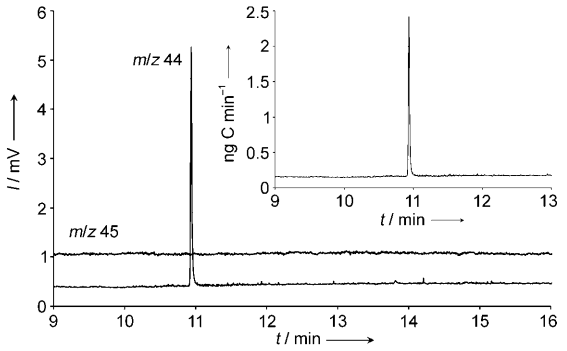

Figure 2. Intensity chromatogram of a single compound (tetradecane) after combustion (*m*/*z* 44), and signal corresponding to the postcolumn spiking (*m*/*z* 45). The inset shows the mass-flow chromatogram obtained after application of the isotope-dilution equation.

these two signals is almost equivalent to the ratio $^{12}C/^{13}C$, since the influence of the oxygen natural isotopic composition is negligible. The prior determination of the ^{13}C concentration (nmol ^{13}C mL^{-1}) in the container by reverse isotope dilution with another pure standard (dodecane) in conjunction with the accurate tracer mass flow provided by the mass-flow controller (mL min^{-1}) gives the tracer mass flow (pmol ^{13}C min^{-1}) at every flow assayed. The stability of the mass flow was tested by connecting the exit of the $^{13}CO_2$-mass-flow container directly to the mass spectrometer and measuring the variation in the tracer isotope ratio *m*/*z* 44/45 in a short-term (GC run, 30 min) and a long-term study (working day, 9 h). The results obtained, 0.0339 ± 0.0002 ($n = 8$) and 0.034 ± 0.001 ($n = 8$), respectively, clearly demonstrated the desired stability. It is therefore possible to carry out a single calibration of the tracer mass flow at the beginning of every working day. Figure 2 shows clearly that the signal measured at *m*/*z* 45 was completely constant along the gradient assayed. Interestingly, the combustion reaction and all the connections required did not lead to significant peak broadening. In fact, the peak width measured at half height (0.02 min) was identical to that observed with conventional GC–MS instruments (i.e. on switching to configuration A).

A combination of the contribution of the solvent tail, the small amount of air that probably enters at every connection between a fused-silica tube and the six-way valve, and the trace of air present in the ionization source does result in significant background noise at *m*/*z* 44 (0.4 mV; Figure 2). This contribution from air affected neither the accuracy nor the precision of the quantification, as it only translated into a relatively constant background in the mass-flow chromatogram (inset in Figure 2). This background turned out to be similar to that observed with other universal detectors, such as FID or MS in the scan mode.

Analytical figures of merit included an adequate dynamic range (assayed up to two orders of magnitude) and a detection limit of 0.8 pg C s^{-1}, which translated into an excellent absolute detection limit of 3 pg of injected tetradecane. This detection limit is three orders of magnitude lower than that observed with postcolumn ^{13}C-isotope dilution and ICPMS (0.7 ng s^{-1}).[8] It also compares very well with

those observed with typical GC detectors (FID: ca. 10 pg C s^{-1}; MS operated in scan mode: 1 pg C s^{-1}). Unfortunately, the precision initially observed (10–15% RSD (relative standard deviation)) was limited by the high uncertainty associated with manual injection into the GC instrument (1 μL or less). Therefore, the use of an internal standard was evaluated. For that purpose, a known amount of an appropriate internal standard was always added to a known amount of the sample, both weighed exactly, so that knowledge of the exact volume of the sample injected was not necessary. An additional advantage of using an internal standard is that the relatively high complexity of the isotope-dilution calculations are reduced to a minimum, as recently described by Heilmann and Heumann for the analysis of sulfur-containing species by postcolumn isotope dilution and GC coupled to ICPMS.[13] Briefly, the mass of C for the internal standard (m_{std}, known) and the mass of C for the species of interest (m_s, unknown) are related to the areas under the corresponding peaks (A_{std} and A_s, respectively), which must be integrated in the same mass-flow chromatogram. The response factor obtained for the internal standard (m_{std}/A_{std}) can then be used to calculate accurately and precisely the mass of C present in each chromatographic peak, simply by assuming an arbitrary tracer mass flow. Of course, such a simplification requires that the tracer mass flow should be completely stable along the chromatogram (a condition fulfilled in our system, as stated above).

The approach was validated by using an *n*-alkane standard mixture. Tetradecane was selected as the internal standard for the computation. Absolute errors in the quantification of the *n*-alkanes from C_{10} to C_{20} ranged from −7 to +5%, and the precision level was lower than 4% RSD ($n=3$) for all compounds (see Table S1 in the Supporting Information). These results demonstrate that, as expected, every *n*-alkane was converted quantitatively into CO_2 in the combustion furnace independently of the length of its chain.

Next, a model mixture containing saturated (undecane, tridecane, and pentadecane), unsaturated (toluene, ethyl benzene, and *o*,*m*,*p*-xylene), and functionalized (butyl butyrate and hexyl butanoate) pure standards (purity > 99%) was created. Again, tetradecane was used as an internal standard. The mass-flow chromatogram and the quantitative results obtained are shown in Figure 3 and Table 1. The recovery for every organic compound analyzed agreed very well with the theoretical values and thus supported the universal character of the quantitative approach (unfortunately, the capillary GC column used was not able to separate *ortho*- and *para*-xylene, so quantitative results for these species are given together).

Finally, to check the performance of the quantitative setup for very complex real samples, we analyzed a diesel fuel directly after a simple dilution with *n*-hexane. The mass-flow chromatogram obtained is shown in Figure 4. In spite of the large number of compounds to be burnt in the furnace, the chromatogram does not show any tailing or degradation. The use of a very low temperature-programming rate (6 °C min^{-1}) in combination with a 60 m long capillary column enabled satisfactory separation of the compounds to be analyzed. In this case, hexyl butanoate was selected as the internal standard, as tetradecane was present in the sample. Because

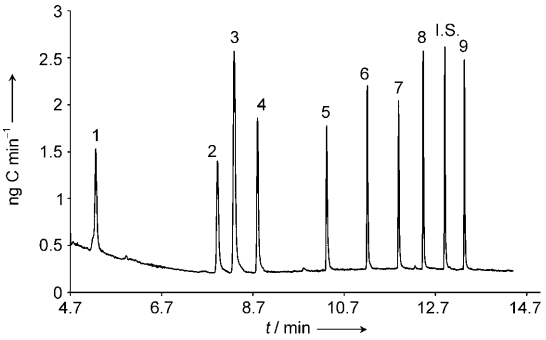

Figure 3. Mass-flow chromatogram of a model mixture containing different families of compounds (BTEX, esters, and alkanes). The identity of the chromatographic peaks (1–9) is given in Table 1.

Table 1: Absolute quantification results for the model mixture.[a]

Peak	Compound	Found [μg g^{-1}]	Expected [μg g^{-1}]	Recovery [%]
1	toluene	5.7 ± 0.3	6.1	93 ± 5
2	ethylbenzene	5.9 ± 0.3	6.1	97 ± 5
3	*o*,*p*-xylene	123 ± 0.7	12.2	101 ± 6
4	*m*-xylene	6.2 ± 0.3	6.1	101 ± 5
5	butyl butyrate	5.9 ± 0.3	5.6	105 ± 5
6	undecane	5.0 ± 0.3	4.7	106 ± 6
7	hexyl butanoate	5.6 ± 0.2	5.6	100 ± 4
8	tridecane	5.3 ± 0.2	5.1	104 ± 4
9	pentadecane	4.8 ± 0.2	4.9	98 ± 4

[a] Tetradecane was used as an internal standard. The uncertainty is expressed as the standard deviation for $n=5$ injections.

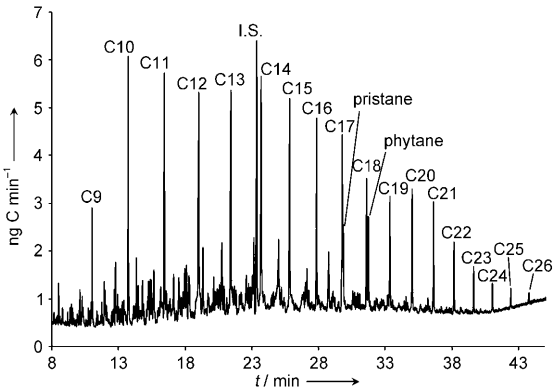

Figure 4. Mass-flow chromatogram of a diesel-fuel sample.

of the species-independent response demonstrated earlier, the most appropriate internal standard can be selected for a particular application. A complementary analysis in the standard GC–MS configuration of the system (position A in the six-port valve, Figure 1) was mandatory to identify the most abundant compounds and check for their peak purity. The main compounds detected and quantified (see Table S2 in the Supporting Information) were C_9–C_{26} *n*-alkanes (Figure 4). Interestingly, some acyclic isoprenoids, such as pristane and phytane, which are typically used to calculate diagnostic ratios in oil-spill investigations, could also be quantified. The other dominant peaks corresponded to

different isoprenoid derivatives (e.g. 2,6-dimethylundecane at 19.3 min and 2,6,11-trimethyldodecane at 23.1 min). Similar quantification by either GC–FID or GC–MS would have required the use of calibration solutions composed of selected compounds to compute the average relative response factors for each group of nearest-eluting species corresponding to the different families of target analytes.[14] Moreover, it would have been necessary to check the instrument calibration for every set of samples analyzed. Thus, time and money are saved with our universal quantitative approach, in which instrument calibration is performed continuously by the tracer flow.

In summary, we have presented the first application of postcolumn carbon-isotope dilution for the successful quantification of any organic species analyzed by GC with a molecular-ion source (electron ionization). The approach, which requires a simple low-cost modification of a standard GC–MS instrument, enables highly accurate and precise quantification of different carbon-containing species eluted from the GC column, without the need for specific standards for each target analyte. Moreover, this instrumental modification can be carried out without any loss of structural information or compound-confirmation capabilities provided by current GC–MS equipment. The expenses associated with the use of isotopically labeled CO_2 for "in situ" instrument calibration are negligible. This feature together with the saving in time and analytical standards (neither calibration nor checking for instrumental-response drift are required) result in a very cost-effective approach. Beyond its use for quantitative quality control in a wide range of standard laboratories, a powerful application of our approach can be foreseen in oil-spill fingerprinting, and in pharmaceutical and environmental analysis, for which the number of organic analytes is so large that it is impossible to have standards for each compound, if they even exist.

Experimental Section

Enriched sodium [^{13}C]carbonate was purchased as a high-purity chemical reagent ($\geq$98%) with an isotopic enrichment of 99%. CO_2 enriched in ^{13}C was prepared as follows: A known amount (ca. 200 mg) of Na_2CO_3 (^{13}C, 99%) was placed in a three-neck round-bottomed flask previously purged with He, and then H_3PO_4 (200 μL) was added to produce $^{13}CO_2$ under an atmosphere of He. A gas-tight syringe was used to extract 2 mL of the tracer and inject it into a 5 L gas container through a septum and an opening valve. The container was then pressurized to 6 atm with He. The standard solution of *n*-alkanes was purchased as a mixture containing 40 mg L^{-1} of each *n*-alkane in hexane, with a purity grade suitable for gas chromatography. The model mixture containing different organic compounds was composed of a BTEX standard mixture (200 μg L^{-1} each, purity $\geq$99%; BTEX stands for benzene, toluene, ethylbenzene, and xylenes) and individual standards of *n*-alkanes and esters (purity $\geq$99.7%). The solvent employed to dilute the different standards and mixtures was *n*-hexane (organic trace analysis grade). The diesel fuel was also diluted by weight in *n*-hexane (ca. 1:800) prior to analysis.

The combustion furnace consisted of a 60 cm × 0.5 mm (inside diameter, i.d.) × 3 mm (outside diameter) ceramic tube packed with copper and platinum wires, placed inside a quartz tube, and heated with a nichrome wire. The furnace temperature was set at 850 °C and regulated by an external temperature controller to within ± 1 °C. The valve employed was a two-position six-way stainless-steel valve with a 0.25 mm bore designed for operation at temperatures up to 350 °C. Fused silica capillaries (0.32 mm i.d.) and appropriate polyimide-coated fused silica adapters were used in the connections of the valve. All analyses were performed with a GC–MS instrument consisting of a Konik HRGC-4000 B gas chromatograph (Konik-Tech, Barcelona, España) coupled to a Konik MS Q12 C series quadrupole mass spectrometer equipped with an electron ionization source.

Received: November 13, 2008
Published online: February 23, 2009

Keywords: analytical methods · gas chromatography · isotopic labeling · mass spectrometry · quantification

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