SemiQuant: New GC/MS Software Approaches to Estimating Compound Quantities

Technical Overview

Introduction

Analysts conducting GC/MS determinations of target compounds in samples will often encounter “interesting” non-target compounds. If these non-target compounds are frequently detected in the analysts’ samples, they become more “interesting”, and some attempts to assign an identity and estimate their concentration is usually undertaken. These compounds may be preliminarily identified by searching mass spectral libraries, or they may remain truly unknowns.

Analysts may be faced with a compound appearing in their samples and in their target compound quantitation list for which a standard has not yet been obtained by the chemist or that is not yet available commercially. Examples for this exist among homologous compound series such as the polynuclear aromatic compounds (PAHs; for example, alkyl phenanthrenes, etc.), the polybrominated diphenyl ethers (PBDEs) or polybrominated biphenyls (PBBs), or in the past, the polychlorinated biphenyls (PCBs). In these cases, the compounds could be tentatively identified by mass spectral features, retention time, and other chemical criteria such as the sample extraction and processing. For these homologous compounds, an estimate of their concentration was often made using the calibrated responses of an existing similar compound. For example, 2-methylphenanthrene amounts might be estimated by the responses for 1-methylphenanthrene, or other hexachlorinated PCBs amounts might be estimated using PCB 153.

Alternatively, Agilent customers downloading eMethods from the Agilent Web site (http://www.chem.agilent.com, Mass Spectrometry, Techniques, eMethods) will find SemiQuant tailored to these approximations. These methods are Retention-Time Locked (RTL) to an Internal Standard or analyte. This internal reference could be used in estimating amounts of the compounds in these methods.

The dangers in this approach are many and should be familiar to all chemists. They include:

- The inherent flaw that the response of the unidentified compound probably does NOT have a response identical to that of the reference compound
- The difference in the response curves’ character (linear versus quadratic) and range (the tentatively identified compound response may be outside the calibrated compound's range)
- Instrumental artifacts, such as issues in injection efficiency and chromatography
- Those involving the relative recoveries or extraction efficiencies of the unknown and target compounds

Cognizant of these dangers but at customers’ requests, the GC/MS ChemStation SW (G1701DA Enhanced mode only) has expanded its feature set to make these estimates more conveniently. These features are referred to as SemiQuant and their general application is described here.

To minimize experimental errors in any implementation, SemiQuant compounds should only be used in RTL methods to ensure that the retention time of the SemiQuant compounds do not change. There are no calibrations updates that correct the retention times used in identification or the ratios of the qualifying ions for SemiQuant compounds.

Terminology

The term “SemiQuant” denotes the estimation of an uncalibrated compound’s amount or concentration by comparison to a calibrated, known compound that has been injected and characterized under the same method of analysis (that is, tune, oven and injection program, ionization mode, etc.). The “semi” prefix is exactly that in meaning; “partly” or “almost”.

SemiQuant Software Menu Items

In the Data Analysis package, under Calibration, two new menu items appear (Figure 1). Additionally in the quantitation database, compounds can be identified as: an Internal Standard, time reference; an Internal Standard, not time reference; a Target compound; and also now a SemiQuant compound (Figure 2).

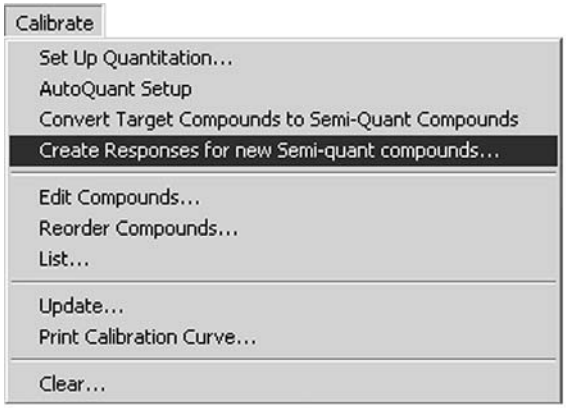


Figure 1. SemiQuant data analysis menu items.

Convert Target Compounds to SemiQuant Compounds

Executing this menu item globally converts all existing Target compounds in the loaded quantitation database to SemiQuant compounds (Figure 2). It does not convert compounds designated as Internal Standards.

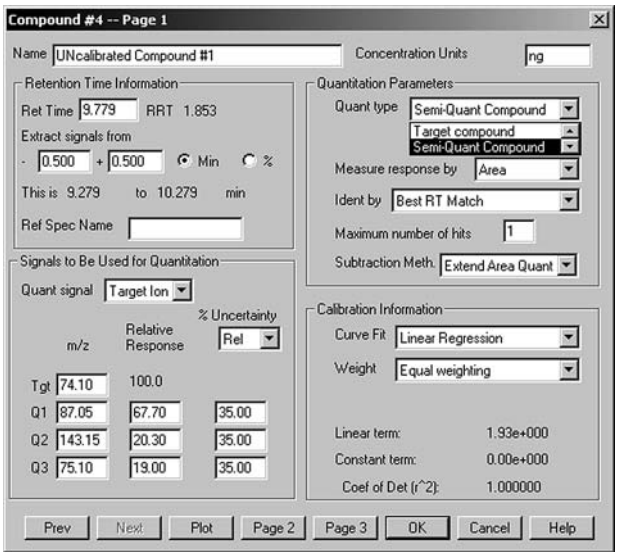


Figure 2. Compound quantitation database – Edit compounds (page 1) SemiQuant compound type.

Create Responses for new SemiQuant Compounds

Executing this menu item brings up two sequential input boxes (Figure 3). The second input box requests the user to set the *relative* response of the SemiQuant compounds to that of their preceding Internal Standard(s). The user can select a multiplier from 0.1 to 10, which is applied to the responses of Internal Standard that precede the SemiQuant compound to calculate the responses of the SemiQuant compound. In other words, if the user selects a multiplier of 2.5, the SemiQuant responses that are generated for the response table (page 3) are those of the preceding internal standard multiplied by 2.5.

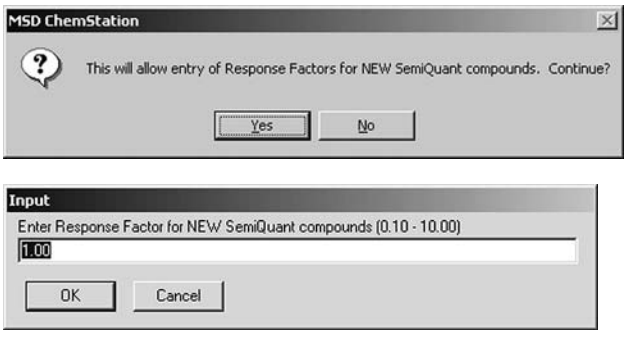


Figure 3. SemiQuant response factor input boxes.

Application in eMethods

For example, if an eMethod is loaded and the user has only injected and calibrated against the Internal Standard (which is typically an RTL locking compound), the other compounds might be considered SemiQuant compounds against the Internal Standard. These would be converted to SemiQuant compounds and then responses would be generated by the user against the Internal Standard responses as described above.

Once the eMethod is loaded, use the Convert Target Compounds to SemiQuant Compounds Menu item described above to convert the method to a SemiQuant method if the eMethod was not created as a SemiQuant method.

Prepare a sample containing the Internal Standards specified by the method and the RTL locking compound at the concentration specified in the quantitation database. Inject this sample and relock the method to achieve the expected retention times. Inject this sample again to verify the RTL lock and update the calibration.

Approaches to Adding a SemiQuant Compound to an Existing Quantitation Database

Users encountering the scenario described in the Introduction who have a quantitation database of calibrated target compounds and wish to obtain SemiQuant estimated amounts for one or more unknown compounds can choose from two approaches for generating SemiQuant compound responses. One approach applies the Internal Standard responses and the other applies the responses of a calibrated target compound.

Applying Internal Standard Response Factors

As above, the Internal Standard in the method is used to generate the response factors.

The procedure for doing this is:

1. Add the compound to the quantitation database using Calibrate\Setup Quantitation. Designate it a SemiQuant compound.
2. Review a calibration report (Calibrate\List\ Calibration Report to determine an estimated response factor for the new SemiQuant compound. If nothing is known about the response of the compound relative to its Internal Standard, a response factor of 1.00 is appropriate. If the compound is a member of a homologous series, note the linear coefficient of a quantitated compound of that homologous series that uses the same Internal Standard.
3. Use the Create Responses for new SemiQuant Compounds items described above to enter the relative response factor for the SemiQuant compound.

Applying a Selected Existing Calibrated Compound's Response Factors

Users can also apply the responses of one of their existing target compounds present in their standards to these unknowns. As previously described, if the uncalibrated compound is a member of a homologous series or resembles another compound of the same class (for example, another organochlorine pesticide or organophosphorus pesticide, etc.), then selecting the response table of the most similar calibrated compound in the quantitation database for the responses of the uncalibrated compound can be done in this way. The calibration table generated from standards for the known compound can be automatically duplicated and applied to those of the SemiQuant compound(s).

The procedure for doing this is as follows:

1. Add the uncalibrated compound to the data analysis method's quantitation database. (Add the compound to the quantitation database using Calibrate\Setup Quantitation.) Indicate that the compound is a SemiQuant compound (Figure 2), add the retention time of the compound and ions that indicate the compound to Page 1.
2. Open Page 2 and in the Type field add UN (for uncalibrated), see Figure 4. In field N4 put the number of the compound in the quantitation database that you want to use as the reference for the responses. Choose a compound that you think is similar in spectral character and elution as the SemiQuant reference. It helps to place the SemiQuant Compound into the table right after the SemiQuant reference.
3. Build the calibration table as usual by building the levels and adding the standards. The SemiQuant compound will automatically have the same responses (as listed on Page 3 of the compound information) as the compound you have designated the SemiQuant reference (for example, see Figure 5)

This approach works for both External and Internal Standard calibrations. Internal Standard corrections for the SemiQuant reference compound (a target compound) are also applied to the SemiQuant compound.

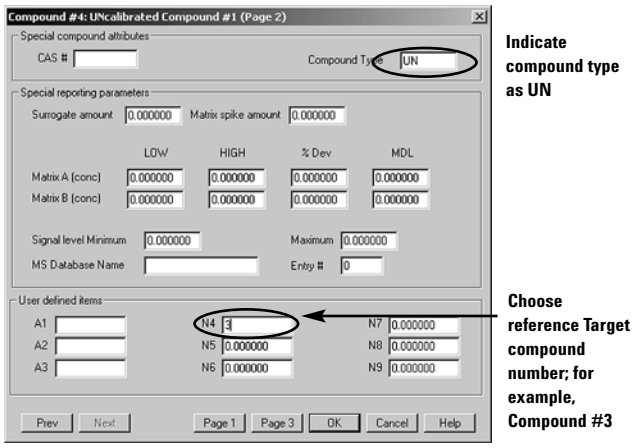


Figure 4. Compound quantitation database – Edit compounds (page 2) uncalibrated compound type.

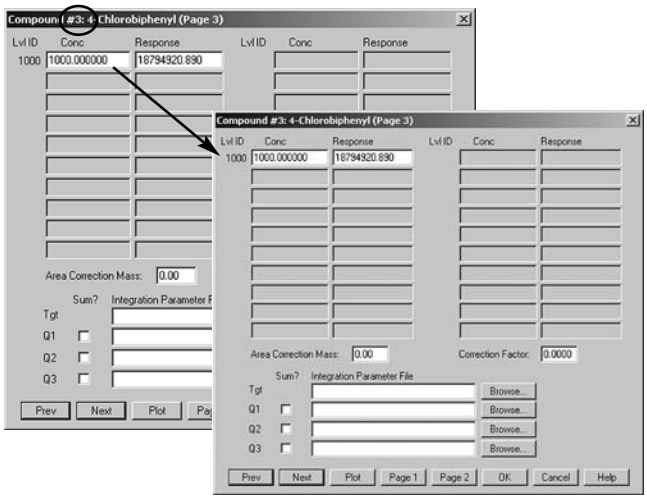
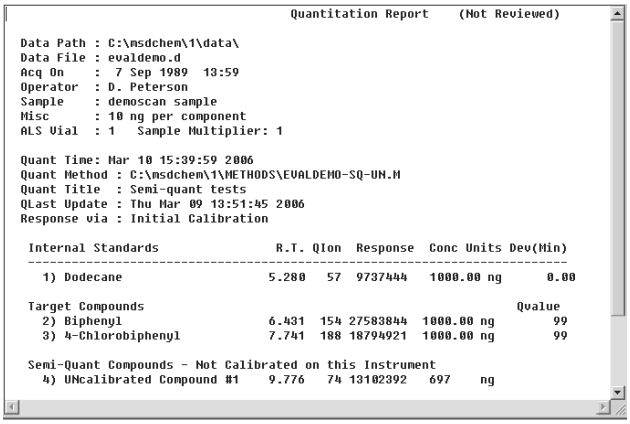


Figure 5. Example for replicating responses for SemiQuant (UNcalibrated) compounds from existing Target Compound responses (refer to Figure 4).

Reporting

A simple example of the SemiQuant report is illustrated in Figure 6. As usual, Internal Standards and Target Compounds are reported, but an additional area at the bottom of the report has been added for the SemiQuant compounds. As is globally true in amounts output by the quantitation calculations, amounts are reported to many more figures than are actually significant, since these numbers may be used in subsequent calculations.



```
                              Quantitation Report    (Not Reviewed)

Data Path : C:\msdchem\1\data\
Data File : evaldemo.d
Acq On    :  7 Sep 1989  13:59
Operator  : D. Peterson
Sample    : demoscan sample
Misc      : 10 ng per component
ALS Vial  : 1   Sample Multiplier: 1

Quant Time: Mar 10 15:39:59 2006
Quant Method : C:\msdchem\1\METHODS\EVALDEMO-SQ-UN.M
Quant Title  : Semi-quant tests
QLast Update : Thu Mar 09 13:51:45 2006
Response via : Initial Calibration

 Internal Standards               R.T. QIon  Response  Conc Units Dev(Min)
 ------------------------------------------------------------------------
   1) Dodecane                   5.280   57  9737444   1000.00 ng       0.00

 Target Compounds                                                    Qvalue
   2) Biphenyl                   6.431  154 2758384   1000.00 ng         99
   3) 4-Chlorobiphenyl           7.741  188 18794921  1000.00 ng         99

 Semi-Quant Compounds - Not Calibrated on this Instrument
   4) UNcalibrated Compound #1   9.776   74 13102392   697    ng
```

Figure 6. Example reporting including SemiQuant compounds.

In situations where it is necessary to have legally defensible results or where critical business or policy decisions need to be made, any qualitative or SemiQuantitative result made regarding a SemiQuant compound should be superseded by the acquisition of an authentic standard of known concentration of the SemiQuant compound. The SemiQuant compound should be converted into a normal Target compound. The method should be calibrated by injecting calibration standards at all relevant calibration levels, ensuring that the retention time, the response factors, and the qualifying ions and their ratios are correct and replaced. Finally, the sample should be re-analyzed by quantitative methods with some attention paid to compound recoveries or other issues in sample preparation and processing.

For More Information

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