



Project Summary

Evaluation of Stable Labeled Compounds as Internal Standards for Quantitative GC/MS Determinations

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During the past 30 years, stable isotopically labeled materials have been used in conjunction with mass spectrometric measurements to provide precise and accurate chemical determinations. Because of this, an investigation into the applicability of stable labeled compounds in the quantification of trace organic environmental pollutants was undertaken. The study focused on the fundamental and practical aspects of priority pollutant quantification in wastewater.

The availability of labeled compounds and the costs associated with using them for routine analyses were evaluated using the volatile, acid, and base/neutral fraction priority pollutants as a target component base. About 75 percent of these compounds are available as appropriate stable isotopically labeled analogs. Incorporation of the stable labeled compounds as part of an analysis protocol is expected to decrease overall analysis costs by 30-70 percent when recommended quality assurance procedures are employed. Approaches to measurement parameter selection were identified which indicate the best ion fragment masses to measure, the best labeled compound spiking concentration, and the most appropriate data reduction methods.

After the fundamental investigations, 20 industrial effluents were analyzed to establish the analytical precision and accuracy of isotope dilution analyses. The samples were also analyzed using a conventional internal standard. This study involved 36 priority pollutants

spiked into the test samples. On the average, isotope dilution yielded accuracies in terms of percent recoveries of 100.0, 99.1, and 98.5 percent for the base/neutral, acid, and volatile fractions, respectively. These values compare with 56.3, 51.6, and 122.6 percent for the same priority pollutants determined using the conventional internal standard quantitation approach. Analytical precision was also better using isotope dilution: 12.1, 19.0, and 9.2 percent standard deviations for the base/neutral, acid, and volatile fractions, respectively, compared with 57.3, 67.5, and 39.5 percent standard deviation for the conventional internal standard data.

This Project Summary was developed by EPA's Industrial Environmental Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The requirement for qualitative and quantitative determinations of trace organic materials in effluent streams was established both by EPA's Environmental Assessment Programs and its response to the 1976 Consent Decree. The use of gas chromatographic/mass spectrometric (GC/MS) analysis for this purpose has received universal acceptance.

One of the most desirable features of GC/MS analysis is its ability to obtain

highly specific qualitative and quantitative analytical information in a single analysis. The qualitative aspects of the technique are well documented. Quantitatively, however, GC/MS results have been disappointing in terms of precision and accuracy. The cost-effectiveness of quantitative GC/MS determinations is also severely impacted when samples, in addition to the original one, must be analyzed to evaluate such things as compound recovery from the original sample matrix. This has been a particular problem in determining priority pollutants in industrial wastewaters, where recoveries have been highly variable. As a consequence, quality assurance procedures involving multiple sample preparations and analysis have been instituted to monitor precision and accuracy. These procedures have increased the cost per analysis by about a factor of two, and do not improve the quality of the data, only check on it.

Similar problems with qualitative and quantitative GC/MS analyses have been encountered in other fields where sample media are extremely complex. Perhaps the most notable of these is pharmacology. The complexity and variation of biological samples, like environmental samples, have resulted in a large number of GC/MS investigations. Because poor analytical results can have an immediate impact on human health in some of these pharmacological studies, reliable high-quality data have been essential. A route to these data has been the use of stable isotopically labeled analogs of the compounds under investigation (target compounds) to act as internal standards for quantitation. With this approach, called isotope dilution, the labeled analog of the target compound is added to the sample prior to preparation. Because the labeled and unlabeled materials are chemically the same compound, the recovery of one can be directly inferred by recovery of the other. Consequently, separate analyses of spiked samples are not necessary and, hence, costs are reduced. It has been demonstrated that this approach to GC/MS quantitation for biological samples provides precisions and accuracies on the order of a few percent over concentration ranges of up to three orders of magnitude. A logical extension of this technology was its application to environmental samples where it was expected to provide equivalent good results.

Results

Results of the study include: the cost and availability of stable labeled com-

pounds, the potential for label exchange, isotope label identification, quality assurance costs, fundamental studies, wastewater demonstrations, and isotope dilution analysis protocols.

Cost and Availability of Stable Labeled Compounds

Because a complete compilation of all compounds of environmental significance does not exist, the organic priority pollutants covered by the Consent Decree were selected as a representative group of compounds in order to perform a meaningful appraisal of cost and availability. These compounds were selected because:

- Priority pollutants cover a broad range of compound types and, consequently, provide a representative base for comparison.
- Matrix effects associated with recovery of priority pollutants during sample preparation are known to exist and to result in limitations to accurate quantitation.
- Priority pollutants are under intense investigation using quantitative GC/MS measurement techniques.

Availability and material costs were assessed on a per-compound basis through discussions with major commercial distributors of stable labeled compounds. Estimates were based on an assumption that 100 μ g of each extractable compound would be added to the sample for each 1-liter sample extracted and 150 ng of each purgeable compound would be added to the sample for each 5-ml sample purged. The material cost estimates per analysis were found to be \$4.34 (1979 dollars).

Deuterium Label Exchange

When deuterated compounds are used as internal standards, the potential for label exchange (deuterium exchange) exists and could lead to errant results. The extent of this potential problem is expected to vary primarily as a function of sample pH. Highly basic or acidic samples would promote deuterium exchange in the same way that they would promote proton exchange; neutral solutions would tend to reduce exchange but not necessarily eliminate it. The potential for exchange with ^{13}C , ^{15}N , and ^{18}O labeled compounds is essentially nonexistent because major molecular bonds would be altered in order for exchange to take place.

Experimental studies for isotope exchange were carried out for the three nonpesticide priority pollutant fractions by spiking solutions of D_2O with naturally abundant priority pollutants and evaluating the mass spectra of subsequent sample extracts for evidence of proton-to-deuterium exchange. Spiked D_2O samples were stored at 4° and 25°C for 0, 48, and 96 hours before extraction. Also, the pD (equivalent to pH for H_2O) was adjusted to give storage conditions of pD's of 2, 7, and 12. In this way, more severe conditions than those encountered in performing a separatory funnel shakeout or continuous liquid-liquid extraction were tested. The results of these experiments indicated that exchange did take place with 6 of the 70 compounds tested. In each of the six instances, however, nondeuterated analogs were available or could readily be synthesized.

Isotope Labeled Detail

Using isotope exchange data and electron ionization fragmentation pattern interpretations, it was possible to identify appropriate labels and label locations for the priority pollutants. For those molecules without specific exchange data, recommendations are based on inference from homologous compounds. For example, all polynuclear aromatic compounds (PNAs) were not available in sufficient quantity to test but, because no exchange of aromatic protons was observed in the PNAs tested, these locations were presumed not to undergo exchange in the untested PNAs. Also, when the precise fragmentation of a molecule was unclear, such as with isophorone, predeuteration or ^{13}C labeling was recommended.

Cost Comparison for Quality Assurance

An estimate of costs associated with the priority pollutant verification protocol was made for a "typical" verification episode. For the 12 data sets associated with the typical verification episode, 36 sample analyses are performed; 24 of these are solely for quality assurance (QA) information. By using isotope dilution where recovery can be evaluated from labeled compound data, sample analyses solely for QA are limited to a precision evaluation; i.e., two additional analyses. The cost per analysis, however, can be expected to increase slightly, primarily due to the reporting costs which now are spread over a smaller number of sample analyses. Consequently, for the 12 sets of data using isotope dilution, 14

sample analyses are performed; 2 of these are solely for QA information. This would correspond to an 87.5 percent reduction in QA costs compared with the existing QA program.

Fundamental Studies

Fundamental studies were undertaken to identify: (1) the best two m/z 's for measurement; (2) the best data reduction methods; (3) ways to evaluate isotopic purity; and (4) the impact of instrument variables on quantification. These are described in detail in the final report and the open literature.

Wastewater Demonstrations

Because the anticipated costs associated with performing priority pollutant analyses using isotope dilution methodology were favorable, a variety of wastewaters and simulated wastewaters were analyzed by isotope dilution (using conventional internal standard methods) and the data compared. The most extensive of these comparisons involved 20 industrial wastewater samples analyzed by two different laboratories; each analyzed each sample spiked and unspiked by both methods. After analyzing the samples, the accuracies expressed in terms of percent recovery and precision were compared for both methods. Fourteen priority pollutants were analyzed in the base/neutral fractions, 8 in the acid fractions, and 13 in the volatile fraction. The mean percent recoveries and mean precisions for these determinations are shown in Table 1.

Isotope Dilutions Analysis Protocols

Because of the highly favorable results generated in the three application studies involving isotope dilution GC/MS quantitation, analysis protocols for that methodology were prepared for the determination of priority pollutants in water and wastewater.

Conclusions

The use of stable isotopically labeled internal standards for quantitative isotope

dilution GC/MS determinations has been shown to be more accurate and more precise than conventional internal standard quantitation approaches for measuring priority pollutants in industrial wastewaters. The isotope dilution method was found to be free of matrix effects which reduce the accuracy of other GC/MS analysis methods and to be equally precise and accurate for measurements in two different laboratories. Finally, incorporation of labeled analogs of compounds of interest into analysis mixtures can be expected to reduce overall analysis cost because quality assurance functions can be incorporated into each sample analysis rather than into the analysis of additional samples.

Recommendations

Although the study clearly demonstrated quantitative isotope dilution GC/MS of great potential value, several aspects of the method are still unclear:

- A full interlaboratory testing of the methods needs to be carried out using blind samples.
- The long-term stability of labeled compounds, especially when contained in spiking solution media, should be investigated.
- The priority pollutant methods should be expanded to include data for all base/neutral, acid, and volatile fraction compounds.
- Isotope dilution should be investigated for potential application to pesticide determination.
- Some available labeled analogs (e.g., methylene chloride- d_2 , 1,1-dichloroethylene- d_2 , and 2,4,6-tri-chlorophenol- d_2) are not ideal labeled analogs; alternatives should be identified.

Table 1. Mean Value for 20 Industries

Fraction	Mean Calculated Percent Recovery		Mean Percent SD	
	624/5 ^a	ID ^b	624/5	ID
Base/Neutral	56.3	100.0	57.3	12.1
Acid	51.6	99.1	67.5	19.0
Volatile	122.6	98.5	39.5	9.2

^aConventional internal standard method.

^bIsotope dilution.

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The complete report, entitled "Evaluation of Stable Labeled Compounds as Internal Standards for Quantitative GC/MS Determinations," (Order No. PB 84-127 711; Cost: \$16.00, subject to change) will be available only from:

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The EPA Project Officer can be contacted at:

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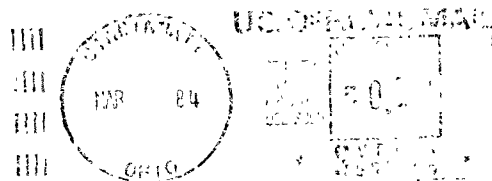
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