

The Preparation of ^{13}C -Labeled Standards of Polycyclic Aromatic Hydrocarbons and other Micropollutants: Synthetic and QA/QC Considerations

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INTRODUCTION:

The technique known as "isotope dilution" analysis has been reported in the scientific literature for more than fifty years, beginning with early work in the biochemical field using the rare stable isotopes oxygen-18 and nitrogen-15 to measure nitrogen and oxygen fluxes in very complex pools. In the 1970s, concerns erupted over levels of "dioxins" in the environment. Because of the acute toxicity of 2,3,7,8-TCDD for some mammals, methods were needed which measured this compound and its analogs among the chlorinated dibenzo-p-dioxins and dibenzofurans at the lowest levels.

Isotope dilution analysis provided an exceptional solution to the problem of quantifying these critical contaminants in various complex environmental matrices. To perform this analysis, an isotopically labeled analog is needed for each target analyte. For 2,3,7,8-TCDD and other target dioxins, carbon-13 labeled analogs were prepared. A precise amount of the C-13 labeled analog is added to the environmental sample prior to extraction and clean-up. The sample is then processed by the laboratory and the final extract is taken on for analysis by GC/MS. Both the native contaminant and the carbon-13 labeled analog are readily identified and quantified by the mass spectrometer. Since the amount of carbon-13 labeled standard is known, a very precise quantitation of the target analyte can be achieved.

REF/QC

For the past twenty years, carbon-13 labeled standards have been used in connection with GC/MS to provide the definitive analysis for dibenzo-p-dioxins and dibenzofurans in the environment¹⁾²⁾³⁾. The success of this method has led to a need for similar analogs of other micropollutants including; polychlorinated biphenyls (PCBs), certain organohalogen pesticides, chlorinated phenolic compounds and polycyclic aromatic hydrocarbons (PAHs). At the very heart of all isotope dilution quantitation is a requirement for fully characterized, quantitative standards of high chemical and isotopic purity. Each group of analytes present particular problems for the standard producer.

In recent years, reports from a number of analytical laboratories pointed out that the usual deuterated PAH standards actually back exchanged during various extractions and work ups⁴⁾. In fact, it has been noted that certain deuterated PAHs will exchange under such mild conditions as standing at room temperature in methylene chloride. The exchangeability problem severely limits the usefulness of these standards for doing GC/MS SIM analysis. As a result, a synthetic program was initiated with the ultimate objective of producing a substantial set of the most useful PAH standards labeled with carbon-13 instead of deuterium.

OBJECTIVES:

The objectives of this study included;

I) Developing cost effective syntheses of carbon-13 labeled analogs of the 16 PAH compounds under consideration.

A) Selection of an unambiguous synthetic route which could be performed in good yields and in a reasonable timeframe. (See Figure 1)

- a) Consider cost of starting materials.
- b) Consider limitations of scale.
- d) Determine which isotopes and how many sites to label in the molecule to minimize costs while still making a useful standard.

II) Produce needed quantities of pure, highly enriched target C-13 PAH standards.

III) Produce quantitative solutions of materials from above.

IV) QA/QC considerations for the final solutions include:

- a) Identification or preparation of reference quality standards of unlabeled, individual PAH compounds.
- b) Verification of identity, isotopic purity and chemical purity of all carbon-13 labeled PAH compounds.
- c) Scrutiny of the final product for ANY presence of native, unlabeled materials.
- d) Preparation of quantitative solutions of the C-13 PAH standards by multiple gravimetric preparations of the pure materials under statistical control.
- e) Demonstration of homogeneity by random analysis of final, ampouled product.
- f) Validation versus Standard Reference Materials whenever possible.
- g) Preparation of useful technical data and material safety data sheets.

SYNTHETIC CONSIDERATIONS:

In general, the synthetic issues were the same ones that face any chemist who is looking at a lengthy and somewhat complex synthesis. One important difference, however, was that the carbon-13 starting materials for each synthesis cost in the range of hundreds to thousands of dollars per gram. Another significant issue was in the scope of the work. In order to have a full set of standards available to the analytical community, it was determined that at least sixteen carbon-13 labeled PAH compounds would be needed. As a result, a great deal of time was spent in planning each synthesis in advance to minimize the number of steps and use the least expensive starting materials. From discussions with a number of analytical groups, it was determined that each standard must have a minimum of 3 atomic mass units (AMU) or 3 carbon-13 atoms per molecule.

QA/QC CONSIDERATIONS:

The analytical issues involved unequivocally establishing the identity of the labeled compound and then demonstrating chemical and isotopic purity. These identifications were accomplished by a series of comparisons of the labeled analog to known standards of the unlabeled compound. The methods used for these comparisons included but were not limited to; absolute retention time by GC/FID, GC/MS, melting point determination, FT-NMR, IR and less frequently UV and HPLC. At the end of this series of determinations, all of the critical QC issues of identity and purity were resolved. As a final part of the quality control, SIM-MS was done to prove that virtually no native material was present in the final labeled analog.

After all analyses are complete and satisfactory, gravimetric preparations are done on each solution, in triplicate. Statistical controls were in place and monitored as

REF/QC

previously reported⁵). The final carbon-13 labeled solutions were directly measured on GC/MS against reference quality unlabeled standards (preferably NIST standards) for quantitative assessment. Statistical controls were in place to monitor any significant deviations. Finally, three (3) randomly selected ampoules from the beginning, middle and end of the ampouling run were pulled and measured against each other as a measure of homogeneity across the packaging process. As always, statistical controls were in place to monitor homogeneity.

CONCLUSIONS:

The utility of carbon-13 labeled standards for the identification and quantitation of micropollutants in the environment is well-established. In fact, the success of the technology as applied to the problem of measuring dioxins and furans at low levels has led to a demand from the analytical community for C-13 labeled standards of other pollutants. Each new standard brings new synthetic and analytical challenges for the standard producer. High costs of starting materials, difficult, small scale syntheses, rigorous requirements by the user community with regard to labeling, purity and quantitation all add to the complexity of standard preparation. A review of the process for developing novel carbon-13 labeled standards is given, with special emphasis on the most recent preparation of carbon-13 labeled PAH standards.

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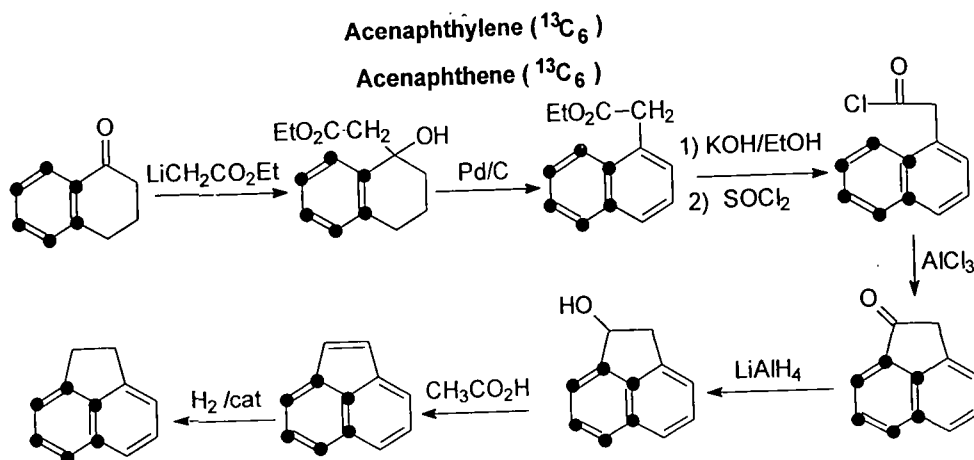


Figure 1

