The Preparation and Validation of Isotope Dilution Standards: A Case Study in PCBs.

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INTRODUCTION:

Isotope dilution is a well established technique for the measurement of pollutants in the environment. This method calls for the addition of a known quantity of the stable isotope labeled analog of the analyte in question. The labeled and unlabeled substrates can be measured precisely in the mass spectrometer. Since the amount of labeled analog is known, the unlabeled pollutant can be quantified precisely. This method has been used extensively for low level quantitation and has been reported on in many articles. [1),2)]

In recent years, analytical groups which have been monitoring total PCBs in the environment have been increasingly interested in doing analyses on specific isomers from this group of more than two hundred. This increased interest centers mostly on the subset of coplanar and mono-ortho substituted "dioxin-like" PCBs which have been shown to have the highest toxicities in the PCB mixtures. In response to this need and in order to bring "state of the art" stable isotope techniques to the area, Cambridge Isotope Laboratories, Inc. has developed and synthesized a wide range of these most interesting PCB isomers, fully labeled with carbon-13.

One of the first problems encountered after the labeled synthesis were completed was the issue of preparation of well-characterized quantitative solutions. This turned out to be a non-trivial problem similar to difficulties encountered in the early preparations of dibenzo-p-dioxin and dibenzofuran reference solutions. A careful examination of commercially available unlabeled standard solutions from three different sources revealed a wide variance in assigned concentrations. Table 1 shows the results of this comparison of solutions of 22 different isomers produced by three different suppliers.

It was determined that the first priority was to develop reference quality standard solutions of the most important unlabeled PCBs, and from these, develop precise assays for the carbon-13 labeled analogs.

Table 1 - Variance Among Commercially Available Unlabeled Standards

Values agreed within:	5%	3 of 22 (14%)
	10%	5 of 22 (23%)
	20%	8 of 22 (36%)
	50%	6 of 22 (27%)

OBJECTIVES:

The objectives of this study included;

A) Preparation of reference quality solutions, under statistical control of unlabeled, individual PCB congeners.

B) Preparation of carbon-13 labeled PCB solutions of reliable quantitative value versus the previously prepared unlabeled analogs.

C) Assignment to each lot of carbon-13 labeled PCB an actual lot analysis versus the reference solution establishing the lot-to-lot variability and reporting it to the user of the standards.

D) Preparation of useful technical data and material safety data sheets.

E) Validation versus Standard Reference Materials whenever possible.

PREPARATION OF UNLABELED REFERENCE SOLUTIONS:

After synthesis and characterization of the pure, unlabeled isomer, triplicate independent weighings are performed on calibrated balances by three staff scientists. Solutions are made of these weighings using redistilled solvents in Class A volumetric flasks. Triplicate, back-to-back injections are made on a Hewlett-Packard 5971 MSD GC/MS. Appropriate statistical controls are applied to this data in order to demonstrate the three solutions are not statistically different (see Figure 1). Validation versus Standard Reference Materials wherever available is also done (See Table 2 and Figure 2).

PREPARATION OF CARBON-13 LABELED SOLUTIONS:

Once the unlabeled reference solutions are in place, the preparation of isotope labeled solutions is made much simpler. A single weighing is carried out and compared directly versus the established unlabeled reference solution. With this, an actual lot analysis is assigned to each batch of isotope labeled PCB isomer (see Figure 3). Ampouling and sealing is done in a manner which maintains the integrity and stability of the standard solutions. Useful technical data and material safety data sheets can be prepared at this time.

CONCENTRATION VALIDATION:

As well as the steps described briefly above, which include validation versus NIST SRMs wherever such materials exist and statistical controls at each step, CIL employed an independent laboratory to make measurements of both the labeled and unlabeled solutions. This laboratory formulated the solutions and carried out the assignment of the lot analyses just as was done in our laboratories. This approach minimized concerns surrounding instrumental or technician-based bias. The agreement was excellent (see Figure 4).

CONCLUSIONS:

Increased isotope dilution measurements of individual PCB congeners in the environment have led to a need for high quality quantitative carbon-13 labeled standards for various PCB isomers. In response to this problem, Cambridge Isotope Laboratories developed procedures to produce , under statistical control, high-quality, reliable unlabeled standards. With these reference standards in hand, isotope labeled solutions are prepared and a lot assay assigned versus the unlabeled reference standard. These standards have compared favorably to available NIST standards. Further the solutions of the se materials are subject to independent formulation and assay by an independent analytical laboratory to assess the final product for in-house instrumental or technician bias. These quantitative unlabeled reference solutions are now used to assay on a lot-by-lot basis the individual carbon-13 labeled PCB standards routinely produced by CIL. These lots are accompanied by technical data and material safety data sheets showing the actual lot analysis of the individual isomers.

Most importantly, by following these protocols, high quality reference standards can be produced which can then be used to manufacture reliable isotope labeled standards for quantitative measurements.

REFERENCES:

1) Pickup, J.F; McPherson, K. *Anal Chem.* **1976**, *48*, 1885.
2) Colby, B.N.; McCaman, M.W. *Biomed. Mass Spectrom.* **1979**, *6*, 225

Table 2. NIST SRM 1585

Chlorinated Biphenyls in 2,2,4-Trimethylpentane

Chlorinated Biphenyl	Concentration	
	µg/g	µg/mL at 23.0° C
4-chlorobiphenyl	43.3	29.9
4,4'-dichlorobiphenyl	9.53	6.57
2,2,4'-Trichlorobiphenyl	3.70	2.55
2,2',5,5'-Tetrachlorobiphenyl	7.72	5.32
3,3',4,4'-Tetrachlorobiophenyl	6.62	4.56
2,2',4,5,5'-Pentachlorobiphenyl	5.24	3.61
2,2',3,4,4',5-Hexachlorobiphenyl	2.37	1.63
2,2',4,4',5,5'-Hexachlorobiphenyl	3.06	2.11

Figure 1.

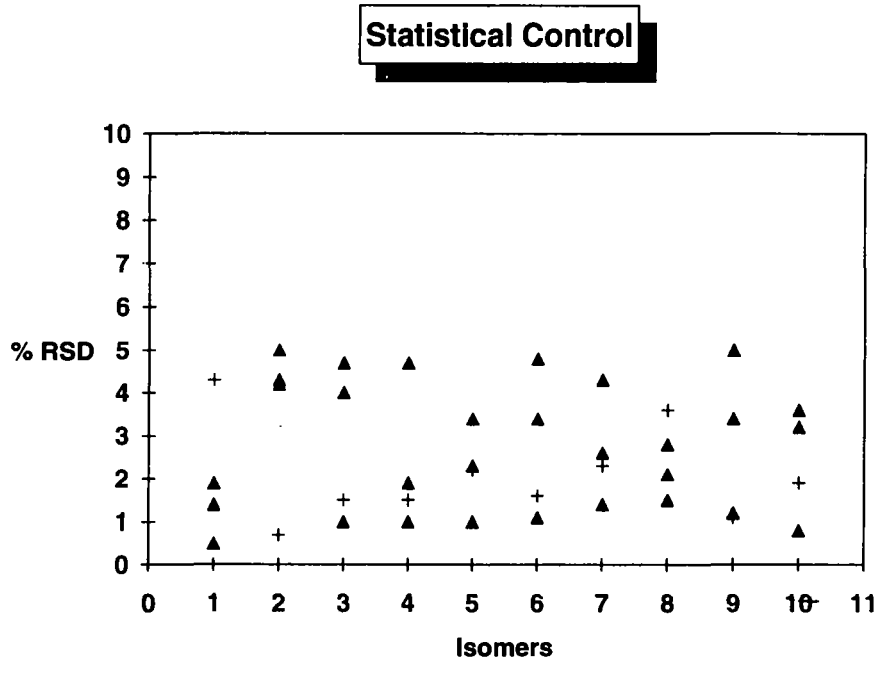


Figure 2

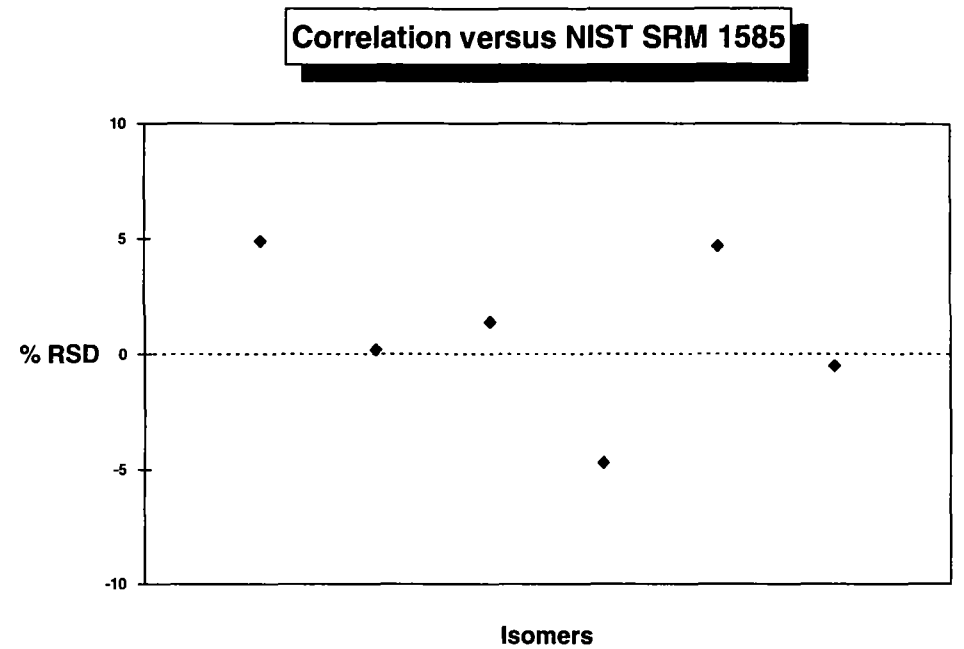


Figure 3

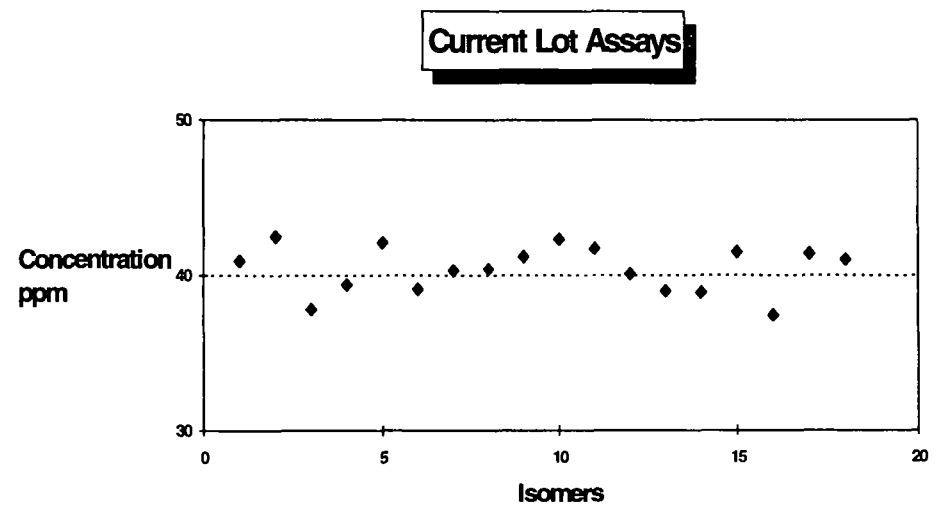
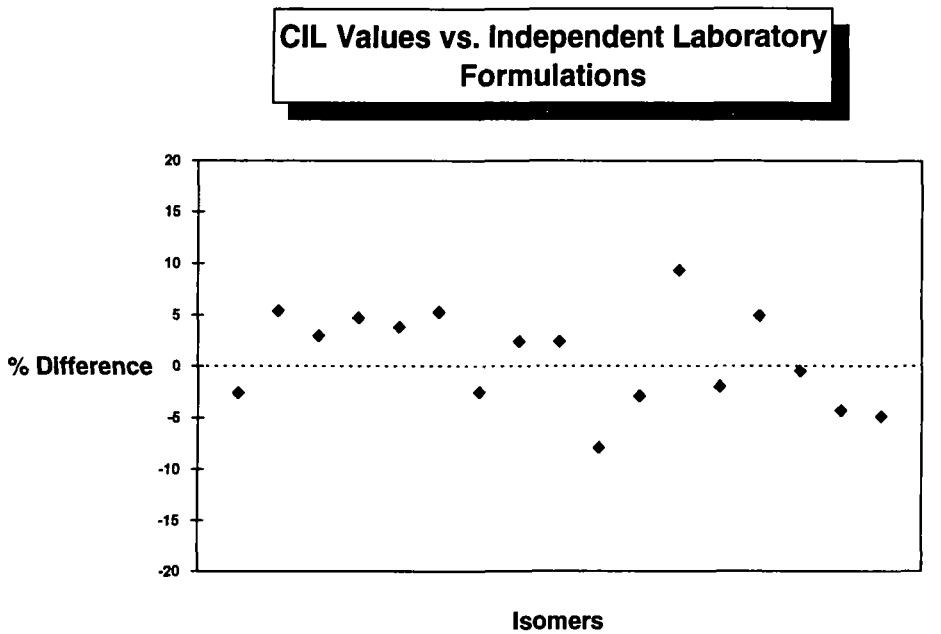


Figure 1


CIL Values vs. Independent Laboratory Formulations
% Difference
20
15
10
5
0
-5
-10
-15
-20
Isomers