SEMI-AUTOMATED EXTRACTION AND CLEANUP METHOD FOR MEASURING PERSISTENT ORGANIC POLUTANTS IN HUMAN SERUM

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Introduction

A method has been developed based on the work by Hovander *et al* (1) for the extraction and quantification of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and persistent pesticides. The updated procedure makes use of laboratory automation to increase sample throughput to 30 samples per day per analyst. Analytical determination of target analytes is performed using gas chromatography isotope dilution high resolution mass spectrometry (GC-IDHRMS).

Materials and Methods

Laboratory automation: Automated liquid handling was performed using two liquid handler Gilson 215, (Gilson Inc, Middleton, WI), equipped with a 402 dual syringe pump and an 818 Automix performing sample mixing by rotation. The first liquid handler was configured for internal standard addition and the second liquid handler was configured for extraction using automated liquid/liquid extraction. The Rapid Trace SPE Workstation (Biotage AB, Upsala, Sweden), was used for the lipid removal step using a two layered silica/silica-H_2SO_4 column packed in a 3cc SPE cartridge.

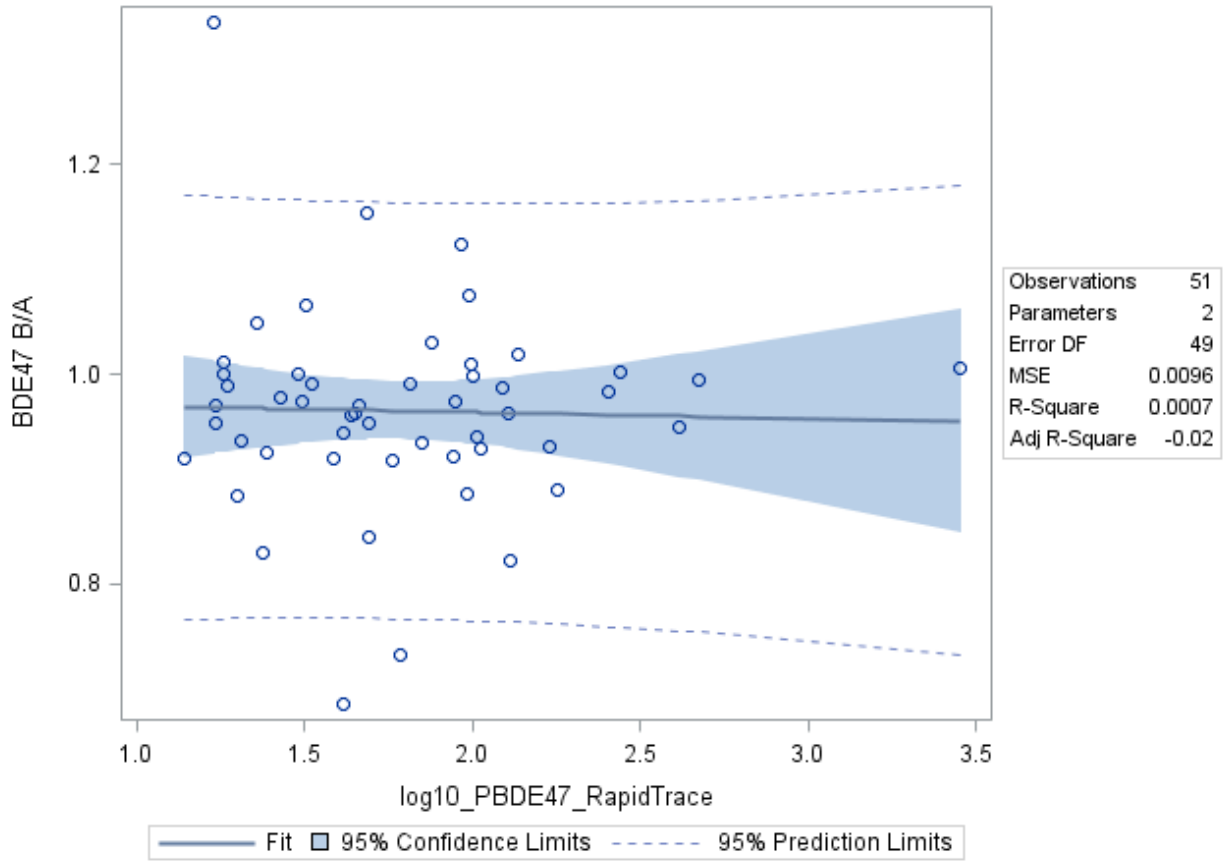
Sample Preparation: Two grams of serum were weighed into 16x100mm culture tubes. These tubes were placed in the automix on the liquid handler for automated internal standard addition and mixing by rotation. Hydrochloric acid (0.5mL, 6M) was then added by hand and the sample tubes were vortexed manually. Then the liquid handler was used to add isopropanol (2.5mL) and 50% methyl-*tert*-butyl ether (MTBE) in hexane (6mL) to the samples with mixing (10min) in between the additions. After centrifugation the organic phase was transferred to a 16x125mm culture tube using the liquid handler. The samples were re-extracted with additional solvent (7mL) and the combined extract evaporated to dryness. Co-extracted lipids were removed using a two layered cleanup cartridge, automated using the SPE workstation. The top layer of the cleanup cartridge was 0.2g of activated silica gel and the lower layer were 1.0g of 33% sulfuric acid in silica gel (w/w) and was eluted with 10mL of 5% dichloromethane in hexane. The samples were evaporated and transferred to a GC vial containing an external recovery standard for analysis by GC-IDHRMS.

Results and Discussion

The analytical measurements of BFRs, PCBs, and persistent pesticides in human serum using the semi-automated liquid/liquid extraction method here presented compares well to an established method based on SPE (2). Agreement with previously used methodology was assessed by analyzing a total of 51 samples using the former SPE method and proposed liquid/liquid extraction method and constructing difference plots (Bland-Altman plots) (Figure 1). These plots indicated no significant bias between the two methods. Calculated recoveries of BDE-47, -153, and -209 in thirty serum samples processed using the SPE method were 63%±4SD, 65%±17SD, and 25%±11SD, respectively. By contrast, the calculated recoveries of the same analytes in 215 serum samples using the semi-automated liquid/liquid extraction method were 64%±8SD, 74%±9SD, and

72%±33SD respectively. The limits of detection for target analytes were in general less than 5pg/mL serum except BDE-209 which had an LOD of 20pg/mL serum.

Figure 1. Bland-Altman plot illustrating the difference between former CDC methodology based on SPE (Method A) and newly developed procedure based on the work by Hovander et al. (Method B). The X-axis in the figure represents the average of Method A and Method B on a logarithmic scale and the Y-axis represent the ratio of Method B over Method A. The regression lines indicates no significant slope and no significant bias between the two procedures, exemplified by the target analyte 2,2',4,4'-tetrabromodiphenyl ether (BDE-47).



References:

1. Hovander L, Athanasiadou M, Asplund L, Jensen S, Klasson-Wehler E. (2000) *J Anal Toxicol.* 24:696-703
2. Sjodin A, Jones RS, Lapeza CR, Focant J-F, McGahee EE, Patterson DG Jr. (2004) Anal Chem. 76: 1921-7