A Laboratory Approach for a Time and Cost-Effective Process for Extraction and Determination of Dioxins / Furans, PCBs and PBDEs

Archer J.C., Shojaee S,

U.S. Food & Drug Administration, Arkansas Laboratory, 3900 NCTR Road, Building 26, Jefferson, AR 72079, jeffrey.archer@fda.hhs.gov

Introduction

The idea of increasing efficiency through reduction of time, resources and expense – regardless of the project - is a desirable result. Historically, the determination of polychlorinated dibenzo-p- dioxins and polychlorinated dibenzo furans (D/Fs), as well as polychlorinated biphenyl (PCBs) and the polybrominated diphenyl ethers (PBDEs) have been both time consuming and expensive. These analyses have taken on a large footprint in multiple laboratories within our FDA facility. We've recently investigated multiple alternatives to increase our program efficiency with fewer resources, somewhat due to attrition within the Agency. Several techniques within our laboratory have been incorporated, such as a matrix independent extraction technique, alternate cleanup technologies, vacuum centrifuge for solvent evaporation and automated data processing steps. Each of these improvements have been documented in the literature[1-5]; however, not all within a single laboratory. The overall method enhancements have already reduced our turn-around time and cost by > 50% each for a batch of 6 samples.

Additionally, these techniques are amenable to the incorporation of Lean Management[6], which involves laboratory layouts that improve workflow cohesiveness and communications while minimizing waste and unwieldly processes. We are expecting additional time saving by consolidating our extraction and cleanup processes into a single laboratory.

Materials and methods

All samples are thoroughly homogenized, then weighed and spiked with ^{13}C-Labeled Internal Standards at least 1-hr prior to extraction, but preferably overnight, in a Hydrotherm^{TM} (HT) beaker (C. Gerhardt GmbH & Co. KG,Oberdollendorf, Germany). The dried filters that are obtained after the automated acid hydrolysis process, HT, are subjected to an abbreviated Soxhlet extraction using hexane in a Soxtherm^{TM} (ST) thimble (C. Gerhardt GmbH & Co. KG), which has been thoroughly described[1]. This 6.5-hour process is followed by extract cleanup methodologies. In this scheme, we have moved to a manual column clean-up using pre-made acid silica gel and carbon columns (CAPE Technologies) following a methodology similar to that described elsewhere[2]. The eluent from the fractionation process is collected in Rocket Evaporator (Thermo Scientific, Waltham, MA) beakers[3] with a GC vial and vial insert directly connected. After the collection of 60 mL Hexane from the acid silica, 20 mL of methylene chloride (DCM) are used per sample for the elution of PCBs (exception of non-orthos) and PBDEs. These are evaporated using the Rocket Evaporator for a 15-minute program using Dichloromethane program. As these are completed, the D/F portion, containing the non-ortho PCBs will be eluting from the carbon column with 20 mL of toluene. The toluene fraction is then subjected to the Rocket Evaporator for 30 minutes using the Toluene method. Each fraction is then ready for instrumental analyses after adding appropriate recovery standard and solvent to proper volume. The analysis of all D/F extracts, including four non-ortho PCBs, are performed using an APGC QqQ MS instrument[4], Xevo (Waters, Milford, MA). The gas chromatograph used was an Agilent 7890 Series (Agilent Technologies) with a 30 m DB5-MS UI (Agilent, J&W, Santa Clara, CA) GC column, a 0.25 mm id deactivated pre-column (Agilent Technologies), and a split split-less 4 mm × 78.5 mm liner (Thermo Scientific, Waltham, MA). The column and guard column were connected with a deactivated press-fit (Restek). Direct isotope dilution was used for reporting all analytes. Additionally, a ^{13}C-labeled Recovery standard containing 1,2,3,4-TCDD and 2,3,4,6,7,8-HxCDD was added to each extract for a final volume of 10 uL with a 1.5 uL injection to determine internal standard recoveries.

If the original extract was expected to contain more than 2.5 g of fat, the PCB / PBDE fraction was subjected to an additional size exclusion chromatography cleanup after evaporation of DCM. Forty microliters of DCM are added to the vial obtained from the Rocket Evaporator for injection onto the HPLC (Agilent Technologies, Santa Clara, CA) – size exclusion column. The 15 mL of DCM collection fraction is then re-evaporated using the Rocket Evaporator DCM method. This extract, after adding recovery standard, is now ready for injection on a GCxGC-ToF MS instrument. The determination of the additional dioxin-like (DL) and marker PCBs were accomplished via direct isotope dilution on a Pegasus 4D-GCxGC-TOFMS (LECO, St. Joseph, MI) using an internal U.S. FDA method, described elsewhere[7]. The injection liner, guard column and press-fits used are identical to those mentioned previously. A 10 m PCB HT-8 primary column (SGE Analytical Science, Austin, TX) with an additional press-fit connection to a 1 m DB-17MS secondary column (Agilent,J&W) was used.

Results and discussion:
The focus of this work is to reduce cost and time involved for POPs determination while reducing the physical footprint for sample preparation, extraction, cleanup, instrumental and final data reduction and evaluation stages. Our sample preparation and extraction processes have evolved from a set of choices that included manual column, separatory funnel, pressurized liquid, sonication to the sole use of automated acid hydrolysis and abbreviated Soxhlet extraction. This has reduced the sample preparation time to simply obtaining a homogenized sample and introducing it into the hydrolysis beaker. The manual involvement for the process to obtain the pure fat extract is less than 2-hours of the 6.5-hour extraction process.

The extract, if fat determination is needed, is heated to 100°C in an oven for 1-hour. If fat determination is not required, the fat extract, containing a small amount of hexane can be directly subjected to a 14 g Acid Silica Gel column followed by a carbon column. Previously, our process involved filtering the fat extracts prior to Gel Permeation Chromatography (GPC) cleanup. After GPC, the extract cleanup was then continued to an automated multi-column technique using a Fluid Management System PowerPrep (Watertown, MA). This move to a manual process has reduced the total organic solvent usage from approximately 1 L to about 0.1L per sample extract cleanup. Meanwhile, although the physical time involvement is similar, the total time has decreased from about 15 hours for 6 extract cleanups to about 3 hours for 6 extract cleanups.

The two fractions for each sample extract are subjected to evaporation using the Rocket Evaporator, with the specified program for the solvent used. Vials and vial inserts that are used with our autosamplers are inserted directly into the Rocket Evaporator vessel and then can be moved directly to the respective instrument for analysis after the addition of recovery standard. The blowdown step for the dioxin fraction with 20 mL of toluene takes 30 minutes, then a recovery standard and additional solvent are added to ready the extract for injection on a GC/MS. Previously the toluene was evaporated using turbo-evaporators, the vessels were rinsed and transferred into the GC vial inserts. The volume in the inserts were subjected to nitrogen evaporator stream. The evaporation process for D/F extract portion would take up to 3 hours for 6 samples compared to 30 minutes. Likewise, the PCB/PBDE fraction is evaporated within 20 minutes versus 1.5 hours for 6 samples.

If the original sample contains more than 2.5 g of fat, the PCB/PBDE fraction is re-constituted in 40 µL of DCM for an additional cleanup on an SEC column using HPLC. This collection is set to collect 15 mL of DCM after the fat has passed through the column in 7.6 minutes. The resulting DCM is then re-subjected to Rocket Evaporation before PCB/PBDE determination via GCxGC-Tof MS. This step adds approximately 3.5 hours to the total process, which adds up to 5.5 hours – approximate 60%-time savings over the cleanup procedure previously discussed at 15-hours.

The final work stages of the process include the instrumentation, data processing and data reduction. The time involved for instrumental work has remained the same, although all D/F determinations are now completed via APGC-QqQ MS instead of magnetic sector mass spectrometer. Also, having incorporated an automated quality control system for the determination of data usability[5], we have reduced our time from 3 hours per batch to 10 minutes.

The diagram shown in Figure 1 illustrates the traditional flow of a sample batch through our POPs laboratory. Depending upon the matrix type, we begin with determining the type of extraction technique to use. If the sample is a liquid, we would proceed to the liquid extraction laboratory with separatory funnels. If the sample is a solid it would go to a pressurized liquid extractor or a manual shaker and centrifuge. After the extraction process we decide whether to complete a fat determination. If a fat determination is needed, each sample is subjected to rotary evaporation until constant weight is obtained. This process may take place in a separate laboratory, causing all extracts to be moved for evaporation. After the fat determination has been completed, a continuation for preparation onto GPC, which includes diluting 1.5 g into 6 mL of DCM in a 10 mL test tube for removing the fat. After GPC, the DCM is evaporated, extract brought up in Hexane for preparation onto column cleanup process. Finally, the D/F determination has historically taken place via GC-HRMS (magnetic sector mass spectrometer) and the PCB/PBDE determinations on GCxGC-ToF MS. The processing takes place via the instrument software and transferred into excel to maintain quality control procedures. By the time this extract has concluded analysis in the POPs laboratory, it will have traveled to a total of 6 laboratories, including instrumental and processing. Additionally, the batch of samples can comfortably be completed and reported in 6 working days, 4 days if urgent at a cost of $250 per sample.

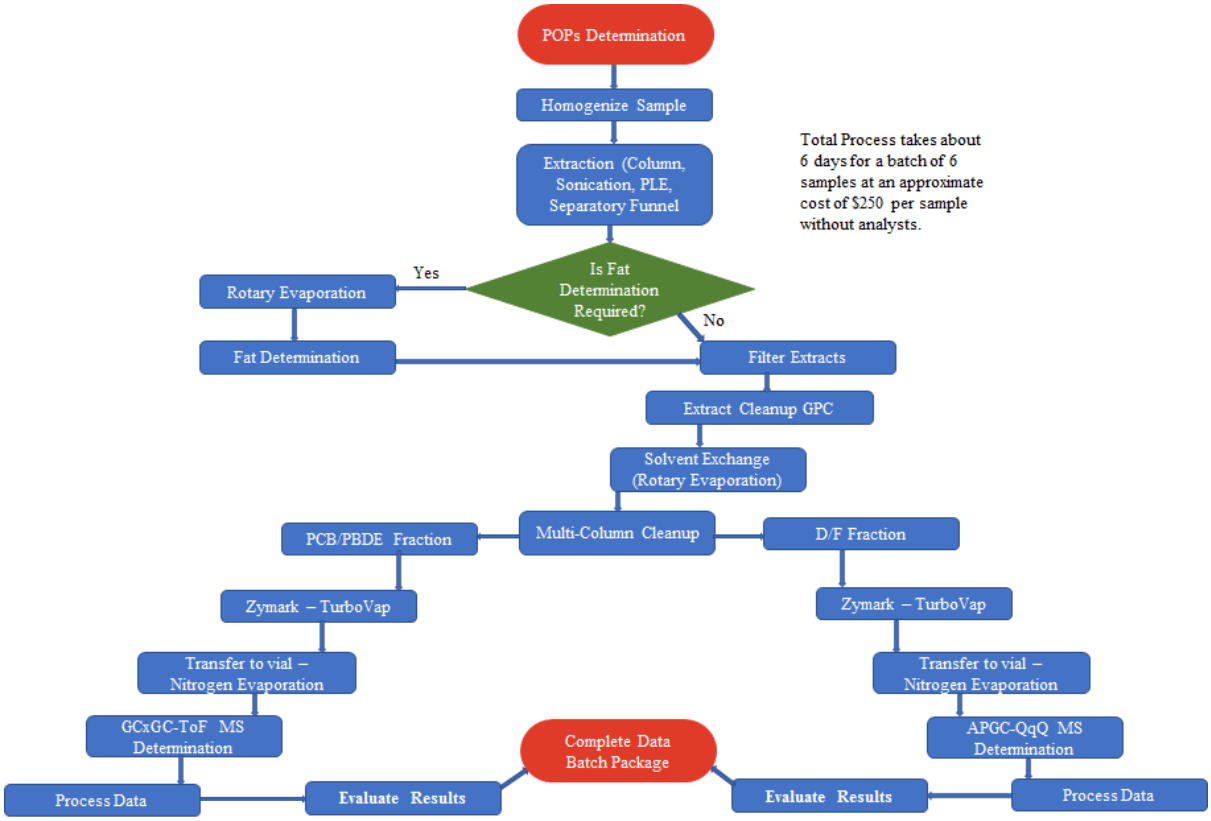

Figure 1. Traditional Sample Flow for POPs Determination at U.S. FDA Field Laboratory.

The flow shown in Figure 2 illustrates our laboratory enhancements and movement toward a Lean program. The first step uses a single extraction technique, regardless of matrix, which readily prepares every extract for fat determination, if needed. A manual column cleanup procedure has been used to reduce the solvent usage and expense, which has also decreased the footprint from the automated system. The manual systems are easily setup within a fume hood. The fraction collection of PCB/PBDE are obtained using 20 mL of DCM per sample and then immediately moved to the Rocket Evaporator for instrument preparation, if the original sample was less than 2.5 g of fat. As this evaporation is complete, the D/F fraction will be completed from the carbon column and ready for evaporation using the Rocket Evaporator. These processes are currently completed in 2 separate laboratories. If more than 2.5 g of fat exist, the dried extracts are diluted in 40 µL of DCM and then cleaned via SEC on an HPLC.

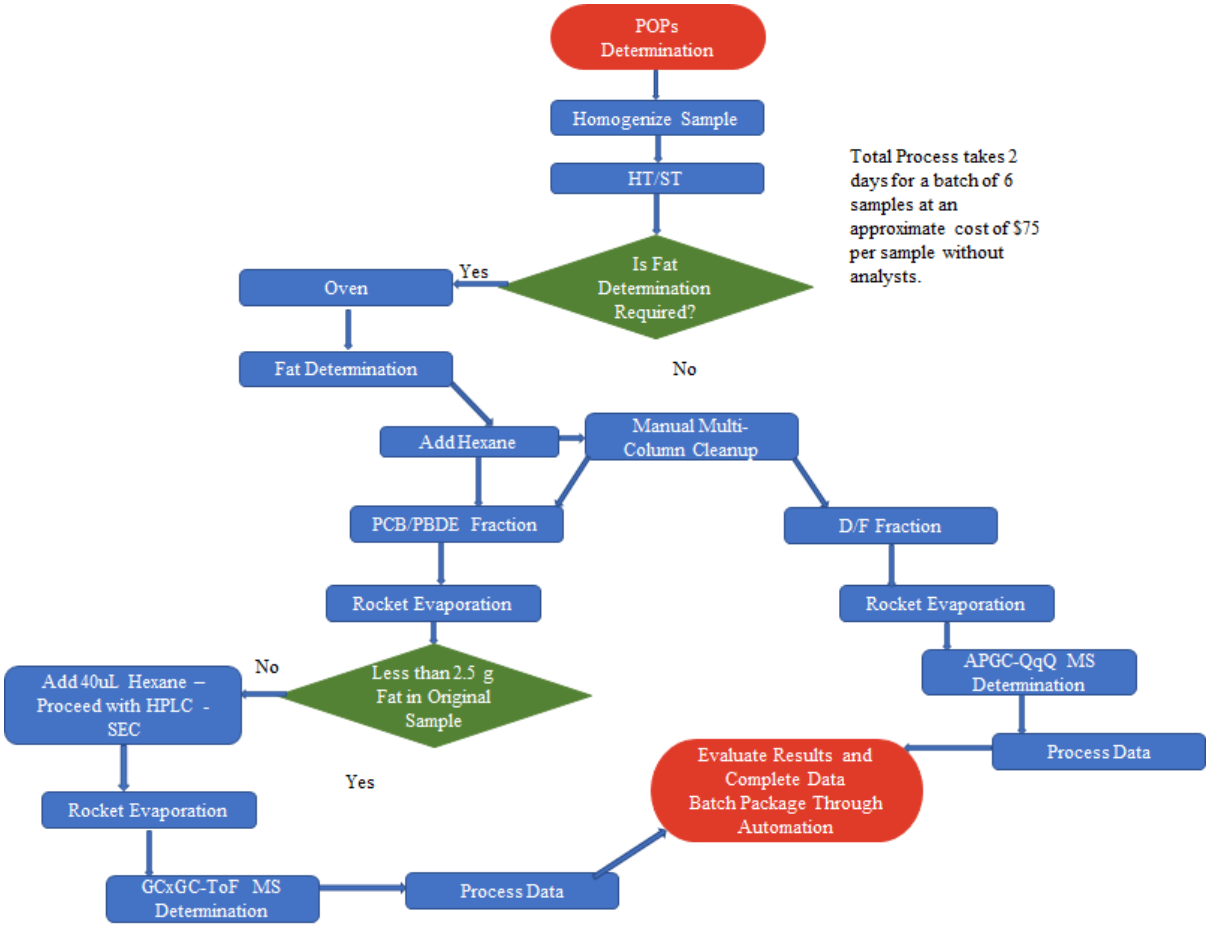

Figure 2. A Lean Approach to Sample Flow for POPs Determination at U.S. FDA Field Laboratory.

Acknowledgements:
The authors would like to acknowledge the U.S. FDA POPs team members who have all contributed to different portions of this work.

Disclaimer:
Mention of trade names or commercial products in the presentation is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Food & Drug Administration. The views expressed in this manuscript are those of the authors and do not necessarily reflect the view or policies of the U.S. Food & Drug Administration.

References:

1. Archer JC, Jenkins RG, et al. (2018); Organohalogen Compd. 80 185-188.
2. Yang S, Reiner EJ, et al. (2013); Organohalogen Compd. 75, 739-742.
3. Hope D, Pond P, et al. (2015); Organohalogen Compd. 77 668-671.
4. Organtini KL, Haimoovici L, et al. (2015) Anal Chem. 87(15) 7902-7908.
5. Archer JC, Moore M, et al. (2018); Organohalgen Compd. 80 269-272.
6. Allen M., Wigglesworth MJ (2009); J. Biomol Screen 14(5) 515-522.
7. Adeuya A, Litman VE (2010); Organohalogen Compd. 72 1125-1128.