The use of non-targeted GC-Orbitrap in the determination of contaminants in human serum

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Introduction

Every day we are all exposed to a wide range of natural and anthropogenic compounds that may impact not just our physical but our mental health. It has been estimated that a woman who wears makeup and other personal care products may introduce up to 500 compounds to the dermal layer every day, and these represent just a small fraction of the total compounds present in the environment. Many of these compounds may find their way into the body, for example, pesticide applied to vegetables and packaging chemicals like phthalates can enter the body via the diet and thus pass through the gut to the bloodstream. Volatile compounds can enter the bloodstream via the lungs, and dust is found to be a potential source of flame-retardants and azo dyes especially for infants who have greater hand to mouth activity[1].

Current studies of human biomonitoring are often limited in that they are targeted for specific compounds. Environmental studies of novel or emerging compounds are often driven by toxicology when a chemical is identified through assays or animal testing to have potential deleterious effects then there is a drive for the study of these compounds in the environment for example this occurred with bisphenol-A. Such a process is logical but the limitation is that toxicology testing is a slow process and environmental monitoring again takes time and requires funding.

Full-scan acquisition for high-resolution chemical identification has in the past been the province of liquid chromatography with either time of flight (TOF) or Orbitrap[2]. The traditional high resolution GC-MS was limited in the resolution making full scan not possible at environmentally relevant concentrations. Recently TOF and Orbitrap have been developed for gas chromatography allowing a new powerful tool for non-targeted GC identification.

Because the GC has not been traditionally used in non-target there is limited understanding of the efficiency of extraction methods for compounds. It is known for example that the USEPA 1613 method is idealized for Dioxins and Furans but it is also understood that compounds such as organophosphate pesticides would not be resolved in this method.

Human biomonitoring is a costly and time-consuming process and there is a big drive to produce biobanks such that we can retrospectively identify exposure and thus develop time trends for exposure. However, these banks have limited samples and they are highly valuable this makes it difficult to obtain material. Given that current high resolution GC and the low sample availability the aim for human biomonitoring should be to achieve the greatest amount of information from the least amount of sample and also to produced full scan acquisition files that could be accessed in the future to investigate emerging compounds rather than access the limited biobanked samples. This project took 5 traditional extraction methods 2 liquid liquid (LLE) extractions and 3 solid phase extractions (SPE) and using a mixture of >250 currently investigated compounds including PAHs, PCBs, pesticides and flame retardants introduced to 0.5mL of male human plasma the extraction efficiency, loss and variability was tested. It then used

samples from oncology patients to validate the use of non-target instrumentation in detection of compounds.

Materials and methods

Triplicate samples of human plasma commercially available were tested via the introduction of a spike of >200 compounds to determine the optimal extraction parameters, (Fig 1).

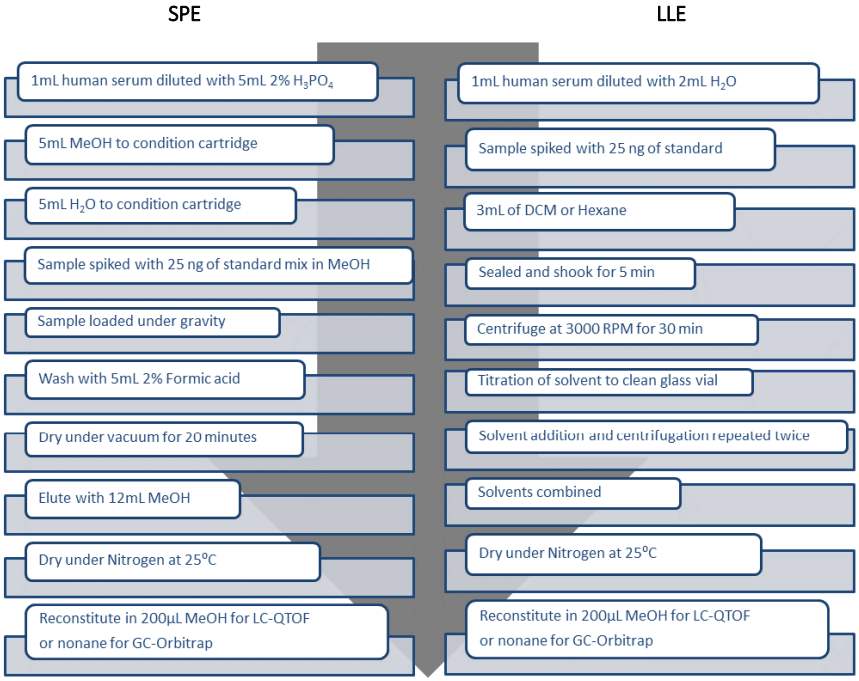

Figure 1: Preliminary extraction schematic for SPE and LLE used in this study

For further validation of the extraction method, 16 samples were used in the initial trial phase for the determination of compounds via-non-target GC. Of these 2 samples contained 200µL of plasma, 3 has 400µL and the remainder 500µL. This indicates the limitations of available sample material for most human exposure assessments. For each sample, 400µL was extracted and the 11 samples with excess material had 100uL pooled. The pooled material was subsequently extracted; 3 spiked samples as matrix recovery and 3 as average concentrations in serum. Human serum AB positive was also used as extraction variance with 5 replications of spiked and non-spiked extracts.

For QA of the potential of contamination via sample vessels and syringes 5 extracts of vials, syringes and needles were performed to identify the primary compounds that may be observed in sera from hospitals but be from external contamination.

Compound identification

Non-Target detection on GC-Orbitrap was done in electron impact (EI) in negative ion mode and chemical ionization in negative (NCI) mode. Quantitative determination of compounds was performed using TraceFinder software with NIST confirmation and peer reviewed literature for compounds where standards were available >200. Suspect screening and feature identification was also performed using TraceFinder, Sieve and R-packages. After the initial trials a further 300 analytes were purchased some GC amenable and others LC amenable for a greater determination of compounds in human sera.

Results and discussion

Target Identification trial extraction

Of the 244 compounds used in the initial trial 156 compounds were positively identified by GCMS-EI +ve using an in-house library in EI mode.

As the method was not optimized for all compounds and many chemicals such as hormones typically only ionize well after derivatization the frequency of detection was not considered an issue as chemicals covered a wide range of physical and chemical properties. Furthermore as compounds from the mix are validated those positively identified are increasing An additional list of more than 400 compounds were subsequently purchased for quantifiable screening in actual samples. Recoveries of the trials of extraction are presented below (Table 1, Fig 2) and though the HLB gave better recovery the variance between samples was slightly greater.

Extraction Method	average recovery	Std dev	compounds
Solid Phase Extraction			
Plexa	42%	8%	84
HyperSep	57%	8%	91
HLB	82%	14%	89
Solvent Extraction			
DCM	78%	13%	86
Hexane	32%	25%	72

Table 1: Recovery of spiked compounds by gas chromatography electron impact in 5 extraction techniques.

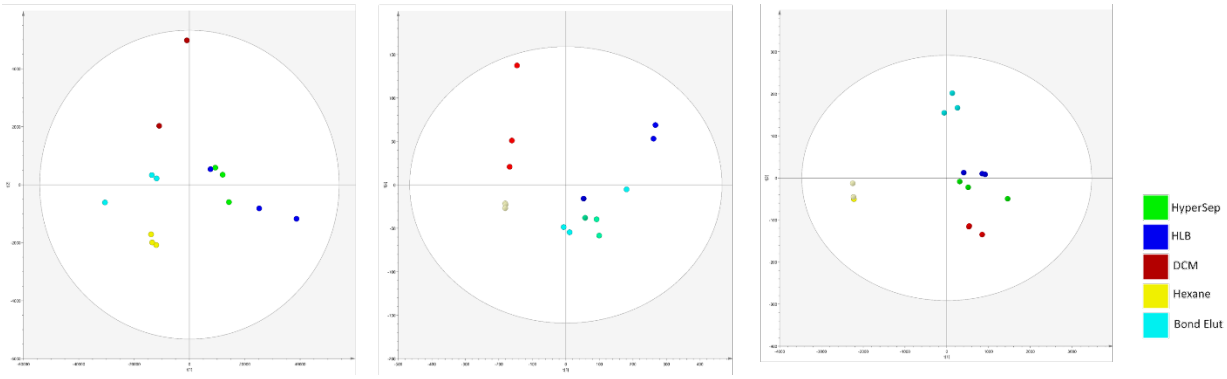

Figure 2: PCA plots of the variance of extraction recovery for 5 extraction methods

As some compounds are more amenable to liquid chromatographic separation and ionization than GCMS further validation of extraction efficiency was by LC-IonFunnel-QTOF the results of which have been submitted for publication[2].

Oncology Trial Sample

The oncology samples (n=16) were ran on GC-Orbitap in EI and NCI to identify compounds in both detection modes. Currently the validation of the identification of compounds is being performed upon these samples but the initial non-target identification of features by PCA is presented here indicating the variance within the samples.

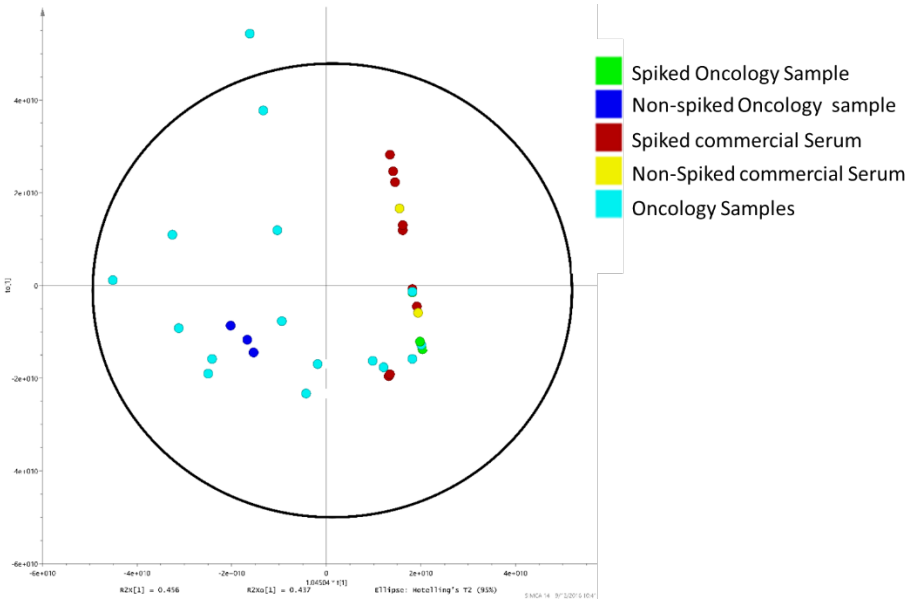

Figure 3: The non-target identification of features in EI of 16 oncology samples by GC-Orbitrap with serum samples both spiked and non spiked from commercially available material and spiked and non-spiked combined oncology serum.

Further Work

The quantitative determination of compounds in the 16 samples are being validated primarily in EI. But as much interest is today on the impact of halogenated compounds such as flame retardants the determination of legacy flame retardants such as PBDEs and more current use FRs are being done via NCI with EI used for compound validation where appropriate. The emerging chemistry tools of high resolution non-target GC mass spectrometry is something that will allow us far greater understanding of the emergence of compounds but also grants us flexibility in our detection in that we are no longer constrained by the limitation of selective monitoring. This project also however understands that the GC-MS cannot be the only tool in the understanding of compound fate and the use of high-resolution liquid chromatography is included in the study to cover the greatest range of analytes.

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