MEASUREMENT OF DIOXIN AND PCB PPT LEVELS IN HUMAN SERUM BY SPE-LC-GC-IDHRMS FOR HIGH THROUGHPUT EPIDEMIOLOGICAL STUDIES.

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Performing measurements at the ultra-trace level is a challenging task. Fortunately, in most cases, today's analytical tools offer the required sensitivity to detect below the ppt level with good reproducibility. To maintain such instrumental LODs (iLODs) while considering complex matrices like human serum, it is mandatory to implement a pre-measurement sample preparation protocol that often consists in a time and resource consuming multi-step procedure. This scenario often results in lengthy processes and very complex analytical QA/QC procedures that are undesirable in large scale epidemiological studies.

A group of 17 2,3,7,8-substituted dioxins (polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)) and 18 selected PCBs (polychlorinated biphenyls) (n_{tot} analytes = 35) are usually targeted in epidemiological studies. Their measurement permits to describe the contamination profile of the specimen and estimate the dioxin-related toxicity in terms of toxic equivalents (TEQs). Because the bio-accumulation of those analytes occurs mainly through the lipid compartment, blood (serum) is commonly the matrix of choice to monitor dioxin levels in humans because of the relatively non-invasive collection of samples, compared to adipose tissue and organ collection.

In the present report, the validation and the routine use of a rapid high throughput multi-step analytical method for the measurement of selected dioxins and PCBs is presented. The extraction of 20ml human serum samples is based on the use of 2g/15ml endcapped (-$Si(Me_3)$) octadecyl solid phase extraction (SPE) disposable cartridges. The clean-up and fractionation in sub-groups of analytes is performed by automated multi-sorbent liquid chromatography (LC). The congener separation and concentration measurement is carried out by gas chromatography (GC) coupled to C^{13}-based isotope dilution (ID) high resolution mass spectrometry (HRMS) using a sector instrument.

Series of 20 samples (1 blank (BC), 1 quality control sample (QC), 18 unknown samples) are processed in batches. Extractions are carried out by a single operator on 2 separate manifolds to facilitate flow control. Sodium sulfate disposable cartridges (2.5g) are placed between the SPE cartridge and the collection tube after vacuum drying (25 min) and prior hexane elution (3 x 5ml). This ensures the complete removal of undesirable water prior to clean-up. The total extraction time is 4h for 20 samples. For clean-up, 6g multi-layer silica (acidic, basic, neutral), 6g basic alumina, and carbon (0.3g) dispersed on Celite (2g) disposable columns are used with the automated system (Power-Prep, FMS Inc., Boston, MA, USA). A first fraction (120ml of hexane-dichloromethane 1:1) containing mono-*ortho*-PCBs (MO-PCBs) and indicator PCBs (I-PCBs) is collected after 45 min. A second fraction (75ml of toluene) containing PCDDs, PCDFs, and non-*ortho* PCBs (NO-PCBs) is collected after 55 min. The entire run time is 75 min, including column removal and system decontamination. Solvent volumes of those 2 fractions are reduced to 500µl using an automated sensor-equipped evaporation device (TurbovapII, Caliper Life Sciences, Teralfene, Belgium). Nonane is added as keeper (90µl and 5µl for fraction 1 and 2, respectively). Remaining volumes of hexane-dichloromethane and toluene are further removed under slight vacuum at controlled temperature using another automated device (RapidVap, Labconco, Kansas City, MS, USA). Lipid content determinations are carried out enzymatically on sub-samples. The total concentration time is 2h for fraction 1 and 3h for fraction 2. Congener separation and measurement is performed during 2 separate GC-IDHRMS runs (one per fraction). Fraction 1 run time is 30min although

fraction 2 analysis requires 1h. Measurements are performed automatically overnight and systematic daily system maintenance is carried out by the operator after each series.

The entire procedure is ISO 17025 and in agreement with EU QA/QC recommendations [1] regarding blank controls, sensitivity, LODs, LOQs, trueness, precision and interlaboratory studies. QC charts based on 'in-house' fortified bovine serum QC pool show random distribution of data points over time and operators, with all data included on a 95% confidence interval. In terms of turnover, once series keep being extracted, analytical results of 20 samples are reported every day, averaging the complete measurement of 100 samples (35 analytes per samples) per week. The cost per sample based on 3000 samples per year for 5 years, including instrumentation paying-off and maintenance, all ID standards, lipid determinations, and employment is estimated to be 550 EUR.

References

1 Commission Directive 2002/69/EC of 26 July 2002, Official Journal of the European Communities, (6.8.2002) L209/5