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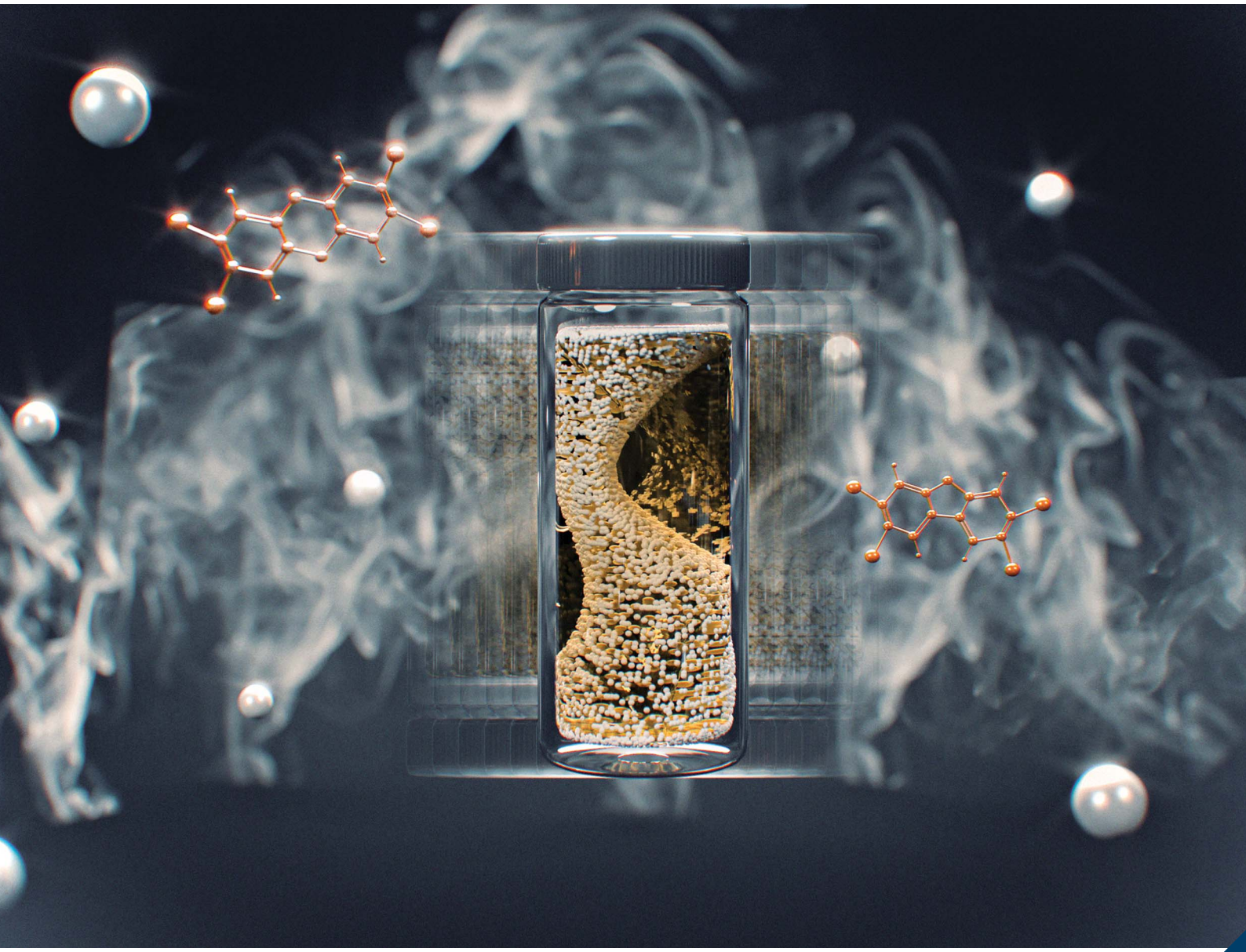

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PAPER
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A novel approach to the extraction and analysis of dioxins and furans sampled onto Amberlite XAD-2 sorbent†

Reinardt Cromhout,[ab] Jean-François Focant[c] and Patricia Forbes *[b]

Despite the efficacy of strong emission control plans that have been implemented the last few decades, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) may still be released from anthropogenic sources such as sinter plants, and municipal and hazardous waste incinerators. Monitoring for PCDDs and PCDFs in gaseous emissions from such facilities is important due to the acute toxicity of these compounds even at trace levels. Currently, most of these samples from the African continent are being analysed abroad at high cost, with the direct consequence that the number of measurements are kept to a minimum. In this context, we developed a more affordable analytical approach for the measurement of PCDD/Fs sampled onto Amberlite XAD-2 sorbent, which relies on a novel extraction, clean-up, and analysis method with the aim of reducing both the cost and the complexity of standard methods while maintaining high quality results. A simple, sequential, 3 hour end-over-end tumbling extraction procedure was developed employing acetone : *n*-hexane (1 : 9) as extraction solvent. This was combined with a dimethyl sulfoxide (DMSO) clean-up to remove aliphatic interferences, prior to direct analysis by gas chromatography triple quadrupole mass spectrometry. The Unites States Environmental Protection Agency Method 23, in contrast, requires a 16 hour Soxhlet extraction with toluene and multiple column chromatography steps. The end-over-end tumbling extraction yielded an average recovery of 79% for PCDD/Fs usually monitored in gaseous samples, whilst an average recovery of 89% was achieved for the DMSO clean-up procedure. In addition, an overall average recovery of 78% and a *Z*-score of −1.1 was obtained using the developed method for the proficiency testing of a solid reference material, proving the method is fit for purpose. It was then successfully applied to the analysis of air emissions from a medical waste incinerator, which further showed that the alternative approach may deliver quality, fast, and cost-effective analysis of gaseous PCDD/Fs sampled onto Amberlite XAD-2 sorbent in a developing country context.

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1 Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) consist of two aromatic rings connected with one or two oxygen atoms with varying levels of chlorination forming 210 congeners (75 PCDDs & 135 PCDFs).[1] PCDD/Fs are lipophilic, therefore the risk of bioaccumulation they pose is high and for that reason PCDD/Fs have received tremendous attention due to their acute toxicity at trace levels.[2,3] Additionally, increased PCDD/Fs levels were successfully correlated with increased fat content and trophic level in Baltic Sea fish species.[4] PCDD/Fs with chlorine atoms in carbon positions 2,3,7,8 present the highest toxicity with 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD being the most toxic, therefore 17 PCDD/Fs congeners are generally tested for by regulatory methods. 2,3,7,8-TCDD has a LD_{50} of 0.6 μg kg^{-1} in male guinea pigs and 155 μg kg^{-1} for rabbits, thus it is clear that toxicity varies for different species.[5,6] Acute toxicity of dioxins and dioxin-like compounds stems from their biological interaction through the aryl hydrocarbon receptor (AHR). Through this interaction, dysregulation was observed in brain metabolomic pathways of Zebrafish, supplementing our understanding of human exposure to PCDD/Fs at trace levels.[7] Based on this, toxic equivalency factors (TEFs) were developed by the World Health Organization (WHO). The purpose of TEFs is to appropriately evaluate the toxicity of compounds relative to that of the most toxic, in this case 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD.[8,9] The WHO released the first TEF values in 1998, which were revised in 2005.[6]

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PCDD/Fs have been found in animal products such as milk, butter, and meat, hence animal feed and food products are routinely tested for PCDD/Fs contamination to limit human exposure.[10,11] Anthropogenic activities are the main source of PCDD/Fs in the environment as they are emitted through incineration of municipal waste, sinter plant operation and hazardous waste incinerators.[12–17] When these sources have ineffective scrubbers and measures to contain PCDD/Fs or to limit their formation, PCDD/Fs will be emitted and subsequently be transported through the atmosphere prior to deposition in sediment and soil.[17,18] Although PCDD/Fs have never been produced on a commercial scale, they may be found as impurities or by-products from the manufacture of chlorinated chemicals such as polychlorinated biphenyls (PCBs), and chlorinated phenols and benzenes.[8,9]

PCDD/Fs have been described as some of the most challenging analytes in analytical chemistry to extract and analyse by Reiner (2010).[19] Extraction of PCDD/Fs is normally very labour intensive when using traditional extraction techniques such as Soxhlet extraction, which is used in most regulatory methods.[19,20] The United States Environmental Protection Agency (US EPA) Method 23 is seen as the gold standard for sample extraction for gaseous PCDD/Fs sampled onto Amberlite XAD-2, whereby the Amberlite XAD-2 sorbent is extracted using a 16 hour Soxhlet extraction process. Various other methods of sample extraction such as pressurised liquid extraction (PLE), microwave assisted extraction (MAE), and supercritical fluid extraction (SFE) have been evaluated as alternative PCDD/F extraction methods to Soxhlet extraction. PLE uses high pressure and temperature to extract PCDD/Fs from solid samples and has been proven to be a good alternative extraction method with acceptable recoveries and reproducibility.[21] However, operational issues with pump cavitation, leaks, blockages, and errors are problematic, which could be detrimental to the extraction efficiency of PCDD/Fs. MAE has been shown to be a very effective and fast extraction technique for PCDD/Fs and PCBs from solid matrices, reducing extraction time significantly compared to traditional Soxhlet extraction.[19,22,23] MAE similarly uses heat generated through microwave energy and pressure to facilitate enhanced extraction efficiency. However, while using MAE automatic pressure venting does occur and could potentially result in loss of PCDD/Fs and other analytes of interest. SFE extracts PCDD/Fs under high pressure and temperature allowing the use of more environmentally friendly CO_2 for extraction for example, but needs extensive optimization and has a risk of cross contamination which could be problematic when analysing unknown samples.[24] Although PLE does have some operational difficulties, it is the most widely used alternative extraction method for PCDD/Fs from solid matrices due to its high level of automation, low solvent usage and comparable extraction recovery compared to traditional Soxhlet extraction.

PCDD/Fs are typically analysed using gas chromatography (GC) coupled to high resolution mass spectrometry (HRMS) which requires very clean sample extracts as trace level quantification is required due to their acute toxicity. US EPA Method 23 uses column fractionation and column clean-up procedures for the purification of sample extracts for PCDDs, PCDFs, PCBs, and polycyclic aromatic hydrocarbons (PAHs) analysis.[20] This sample clean-up procedure is laborious and uses large volumes of solvent, which make it challenging to implement in countries that have to develop routine emission monitoring plans and furthermore it is not in line with current basic green chemistry practices.[20]

A simple sample clean-up technique was developed by Kitamura *et al.* (2004) to clean PCDD/Fs from high lipid content biological samples using dimethyl sulfoxide (DMSO) and acetonitrile partitioning of PCDD/Fs to simplify traditional complex sample clean-up procedures.[25] These extracts are further cleaned using column chromatography, utilising multilayer silica gel-activated carbon.[25] Although this technique still uses a column clean-up procedure, it only uses one opposed to multiple in US EPA Method 23. This limits sample handling and reduces the risk of sample losses and has a faster extraction time of 2–3 hours. Abad *et al.* (2000) successfully evaluated a sample clean-up system which automated the extensive sample clean-up required for PCDD/F testing using GC-HRMS.[26] This system is well suited for the routine testing of samples, however it employs the environmentally unfriendly Soxhlet sample extraction technique and it is based on expensive flow management systems (FMS). A fully automated extraction and sample clean-up system was subsequently developed by Focant *et al.* (2002) using PLE integrated to an automated multi-column clean-up system.[27] This replaces all laborious sample extraction and clean-up steps, however, the system is complex and is also based on an expensive instrument which hinders suitability in a developing country context. Fiedler *et al.* (2022) showed that there is a lack of laboratories in developing countries, such as those in Africa, for the analysis of dioxin-like persistent organic pollutants, therefore highlighting the need for a new approach to enable developing countries to conduct analyses for these compounds on the African continent.[28]

The aim of this study was therefore to investigate an alternative means for the extraction of PCDDs and PCDFs from a solid matrix, namely Amberlite XAD-2 sorbent, using an end-over-end extraction as an alternative to the reference US EPA Method 23 and other automated systems. Furthermore, the optimization of a DMSO clean-up procedure was carried out to allow for direct injection into a gas chromatograph-triple quadrupole mass spectrometer (GC-TQMS) system, a proven alternative to HRMS.[29] This overall combined procedure was tested in terms of robustness and repeatability, to determine its suitability as an alternative PCDD/F analytical approach to facilitate effective emission control in developing countries.

2 Experimental methods

2.1 Chemicals, standards and samples

Solvents namely, dimethyl sulfoxide (DMSO, 97.7%), *n*-hexane (Hex, 97%), toluene (Tol, 99.9%) and acetone (Ace, 99.8%) were all HPLC grade and were supplied by Honeywell, Riedel-de Haën (Seelze, Hanover, Germany).

A PCDD/F native calibration standard EPA 8290 STN containing seven PCDDs (2,3,7,8-tetra-CDD; 1,2,3,7,8-penta-CDD;

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1,2,3,4,7,8-hexa-CDD; 1,2,3,6,7,8-hexa-CDD; 1,2,3,7,8,9-hexa-CDD; 1,2,3,4,6,7,8-hepta-CDD; 1,2,3,4,6,7,8,9-octa-CDD), and ten PCDFs (2,3,7,8-tetra-CDF; 1,2,3,7,8-penta-CDF; 2,3,4,7,8-penta-CDF; 1,2,3,4,7,8-hexa-CDF; 1,2,3,6,7,8-hexa-CDF; 1,2,3,7,8,9-hexa-CDF; 2,3,4,6,7,8-hexa-CDF; 1,2,3,4,6,7,8-hepta-CDF; 1,2,3,4,7,8,9-hepta-CDF; 1,2,3,4,6,7,8,9-octa-CDF) with nominal concentrations of 1.0; 2.5; or 5.0 μg mL^{-1} respectively (this standard contains analytes at variable concentrations, for tetra-CDD/Fs, penta to hepta-CDD/Fs and octa-CDD/Fs, respectively). In addition, an EPA 23 internal standard stock solution (ISS) containing $^{13}C_{12}$-PCDD and $^{13}C_{12}$-PCDF labelled internal standard, $^{13}C_{12}$-PCDDs ($^{13}C_{12}$-2,3,7,8-tetra-CDD; $^{13}C_{12}$-1,2,3,7,8-penta-CDD; $^{13}C_{12}$-1,2,3,6,7,8,-hexa-CDD; $^{13}C_{12}$-1,2,3,4,6,7,8-hepta-CDD; $^{13}C_{12}$-1,2,3,4,6,7,8,9-octa-CDD) with nominal concentrations of 1.0 μg mL^{-1} and 2.0 μg mL^{-1} respectively (tetra-$^{13}C_{12}$DD to hepta-$^{13}C_{12}$DD and octa-$^{13}C_{12}$DD, respectively) and four $^{13}C_{12}$-PCDFs ($^{13}C_{12}$-2,3,7,8-tetra-CDF; $^{13}C_{12}$-1,2,3,7,8-penta-CDF; $^{13}C_{12}$-1,2,3,6,7,8-hexa-CDF; $^{13}C_{12}$-1,2,3,4,6,7,8-hepta-CDF) with a concentration of 1 μg mL^{-1} for all analytes was used. The EPA-23 surrogate standard stock solution (SSS) employed contained $^{37}Cl_4$-2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4,7,8-HxCDD with nominal concentrations of 1 μg mL^{-1}. Lastly, the EPA-8290 recovery standard solution (RSS) used contained $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD with nominal concentrations of 0.5 μg mL^{-1}, respectively. All standards were supplied by Wellington Laboratories (Guelph, Ontario, Canada). Four calibration standards were prepared from these in DMSO for tetra-CDD/Fs, penta to hepta-CDD/Fs and octa-CDD/Fs, as shown in Table S4† using EPA 8290 STN for method validation. PCDD/F native calibration standard EPA 8290 STN was supplied in a mixture of nonane and toluene, thus these solvents were evaporated under a slow stream of nitrogen and DMSO was added.

In the absence of an available Amberlite XAD-2 proficiency testing (PT) sample for PCDD/Fs, a solid PT sample, namely SPE016-10G was used, with concentrations of PCDD/Fs ranging between 90 and 1251 pg g^{-1} on a soil matrix, as supplied by Merck-Supelco (St. Louis, Missouri, USA).

Three real stack emission samples from a medical waste incinerator facility in Africa were sampled using an ISO 17025:2017 accredited US EPA Method 23 technique. The Amberlite XAD-2 sorbents employed were spiked with 10 μL EPA-23 SSS labelled surrogate standard prior to stack emission sampling. The expected concentration of each of the surrogates in the final extracts was 100 μg L^{-1}.

2.2 Analytical instruments and method

Analyses were conducted using a Bruker Scion 436 TQMS equipped with a 1079 programmable temperature vaporization (PTV) injector (Bruker Daltronics, Fremont, California, USA). Liquid extracts, 2 μL, were injected splitless into the PTV, onto a deactivated glass wool liner (3.4 mm inner diameter (ID); 5.0 mm outer diameter (OD); 54 mm length) supplied by Restek Corp. (Bellefonte, Pennsylvania, USA) at 300 °C. Chromatographic separation was achieved by a BPX-DXN column (60 m × 0.25 mm i.d. × 0.25 μm df) supplied by Trajan-SGE (Melbourne, Victoria, Australia). GC programming was set at 48 °C for 1 min, increased to 300 °C at 10 °C per min and held for 16 min for a total run time of 42.20 min with a He flow rate of 1.8 mL min^{-1}.

The transfer line and ion source temperatures were set at 300 °C. Electron ionisation (EI) was performed at 70 eV using multiple reaction monitoring (MRM) with Ar at 1.5 mTorr as collision gas. MRM ions used for calibration and quantification of PCDD/Fs are provided in Tables S1–S3† in the ESI.†

2.3 Extraction using pressurised liquid extraction (PLE)

A Dionex 200 accelerated solvent extractor (ASE) (Dionex, Sunnyvale, California, USA) was used for pressurised liquid extraction (PLE) to establish baseline extraction of PCDD/Fs from Amberlite XAD-2 sorbent.

A mass of 10.0 g Amberlite XAD-2 sorbent was spiked with 1 μL EPA 8290 STN, native PCDD/F standard at a concentration of 1.0; 2.5; or 5.0 μg mL^{-1} and further extracted using *n*-hexane : acetone (1 : 1). The PLE program parameters were as follows: pressure of 1500 psi, 75 °C, 5 min heat and static cycles and with a total of two cycles.[30] The extract was collected in a 60 mL EPA vial (CNW Technologies/ANPEL Laboratories Inc, Shanghai, China) filtered and dried using anhydrous sodium sulphate (City Star Holdings, Johannesburg, South Africa, 99%) and concentrated in a TurboVap LV evaporator (Zymark, Massachusetts, USA) at 40 °C and 2 psi using compressed air, before being transferred to a Kuderna–Danish (K–D) concentration tube. Extracts were concentrated to 500 μL using a slow stream of nitrogen in a custom-built system. It was then transferred to an amber 2 mL GC vial with a polydimethylsiloxane (PDMS) septum (ALWSCI Technologies, Zhejiang, China). The PLE extraction was repeated in duplicate to evaluate repeatability.

2.4 Extraction of PCDDs and PCDFs from Amberlite XAD-2 sorbent using a novel end-over-end tumbling approach with various solvents

A custom-built end-over-end tumbler normally used in the leaching of samples for the assessment of waste for disposal purposes was customized to fit a 60 mL Environmental Pollution Agency (EPA) standard vial. The tumbler operates at approximately 36 fixed revolutions per min (rpm).

Spiking of Amberlite XAD-2 was conducted in the same manner as in Section 2.3. A volume of 30 mL solvent was then added to the Amberlite XAD-2 sorbent. The vial was intentionally not filled to the brim to create a turbulent environment when the extraction vial was rotated in the tumbler end-over-end. *n*-Hexane; *n*-hexane : acetone (1 : 1); toluene; and acetone : *n*-hexane (1 : 9) were the solvents evaluated for the extraction of PCDD/Fs from Amberlite XAD-2 sorbent. These solvents were replaced every hour for 3 hours. Thereafter the combined extracts were filtered and dried using anhydrous sodium sulphate prior to concentration, as described in Section 2.3.

2.5 Partitioning of PCDDs and PCDFs into DMSO

Variable volumes of *n*-hexane (50; 100; 250; 500; 1000 and 5000 μL) were similarly spiked with 1 μL of EPA 8290 STN native

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PCDD/F standard. A volume of 500 μL of DMSO was added to variable volumes of *n*-hexane, and this was vortexed for 1 min and repeated twice in a 2 mL clear GC vial (ALWSCI Technologies, Zhejiang, China). Clear GC vials were necessary to clearly observe phase separation. The mixture was centrifuged (MSE, Crawley, West Sussex, England) for 1 min at 1000 rpm to separate *n*-hexane and DMSO layers. The DMSO extract was separated from the *n*-hexane using a 1000 μL liquid tight glass syringe and transferred to an amber 2 mL amber GC vial with a PDMS septum for storage.

A volume of 5 μL of EPA-23ISS was added to a 2 mL amber GC vial, and dried under a slow stream of nitrogen for 30 min or until visibly dry. The DMSO extract (100 μL) was then added to the 2 mL amber GC vial containing the dried EPA-23ISS, and ultrasonicated for a minimum of 10 min. This DMSO extract containing EPA-23ISS was transferred to a 2 mL amber GC vial with a 200 μL spring loaded insert (ALWSCI Technologies, Zhejiang, China) and was analysed using the GC-TQMS method described in Section 2.2. This was repeated for each variable *n*-hexane volume (Fig. S1).†

2.6 Extraction and analysis of PCDD/Fs from a solid proficiency testing (PT) sample

A sample of 10.0 g of PT soil was weighed and added to a 60 mL amber EPA vial. A mixture of acetone : *n*-hexane (1 : 9) was prepared followed by the extraction procedure described in Section 2.4. Extracts were filtered and dried using anhydrous sodium sulphate and were washed with 10 mL acetone : *n*-hexane (1 : 9) between solvent replacements. Finally, a wash of 20 mL acetone : *n*-hexane (1 : 9) was repeated twice. The sample extract had a cloudy appearance and moisture was suspected. The sample was thus filtered again using anhydrous sodium sulphate as described in Section 2.3 and concentrated to dryness using a slow stream of nitrogen. This concentration step was necessary to optimise sensitivity due to the low unknown spiked concentration of the PT. The dried extract was then redissolved using 25 μL *n*-hexane, whereby the sides of the K–D tube were rinsed numerous times to ensure all analytes were redissolved from the dried extract.

The resulting PT extract was cleaned twice using 250 μL DMSO as detailed in Section 2.5 using the optimised volume of DMSO which was ten times higher than the non-polar solvent, in this case the *n*-hexane PT soil extract of 25 μL. A distinct upper non-polar organic layer of *n*-hexane, could be observed above the DMSO layer in each extract (Fig. 1), showing the effectiveness of the DMSO separation in extracting the target analytes from other unwanted components in the sample extract during the clean-up procedure. The DMSO cleaned extract would contain PCDD/Fs, PCBs and PAHs present in the sample. The combined DMSO extracts were subsequently analysed by GC-TQMS as described in Section 2.2.

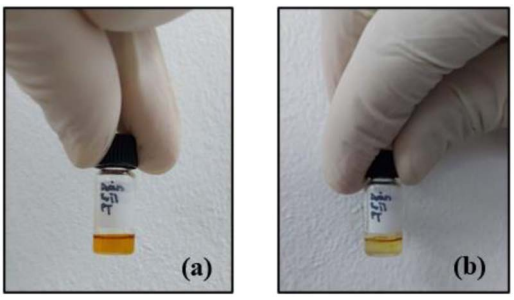

Fig. 1 Image of the extract following the 1st DMSO clean-up (a) and after the 2nd DMSO clean-up (b). A dark non-polar layer is visible on top of the polar aprotic DMSO.

2.7 Extraction and analysis of PCDD/Fs sampled from a medical waste incinerator onto Amberlite XAD-2 sorbent

The extraction technique was adapted from Sections 2.4 and 2.5 to accommodate the high moisture content expected in these real samples and to lower the PCDD/F quantification limits by reducing the volume of DMSO to cater for variable volumes of sampled air and analyte concentrations.

The Amberlite XAD-2 was removed from the stack emission sampler housing described in US EPA Method 23. The sorbent was initially extracted using 30 mL of acetone spiked with 5 μL EPA-23 ISS to remove the large amounts of moisture present in the samples. Extractions were performed for 1 hour using the end-over-end tumbler described in Section 2.4, followed by a 3 hour extraction replacing the solvent every hour (Section 2.4) using acetone : *n*-hexane (1 : 9). These extracts were combined and dried using anhydrous sodium sulphate. They were then concentrated in a 60 mL glass tube with a tapered tip to 100 μL using an evaporator at 40 °C and 2 psi. The tapered tube was then rinsed first with 2 mL *n*-hexane and concentrated to 100 μL, then repeated with 500 μL *n*-hexane, which was again concentrated to 100 μL. This sample extract was transferred to a 4 mL clear GC vial (ALWSCI Technologies, Zhejiang, China), and the tapered tube was subsequently rinsed with 100 μL of *n*-hexane which was added to the vial to provide a sample extract volume of 200 μL. This extract was cleaned twice using 2 mL DMSO as detailed in Section 2.5 maintaining a 1 : 10 ratio of acetone : *n*-hexane (1 : 9) to DMSO. These extracts were combined and 10 μL EPA-8290 RSS was added and concentrated to 40 μL using an evaporator at 60 °C and 5 psi. EPA-8290 RSS was used to evaluate the recovery of the surrogate standards (EPA-23 SSS) spiked prior to stack emission sampling.

3 Results and discussion

3.1 Gas chromatography (GC) and mass spectrometry (MS) performance

Calibration and linear range determinations were done as described in Section 2.2, and correlation coefficients (R^2) for all 17 measured native PCDD/Fs are shown in Table S5,† whilst an example of a linear calibration curve for 1,2,3,7,8-PeCDD is provided in Fig. S2.† Furthermore, a chromatogram of the standard, including all native PCCDD/Fs and labeled internal standards, is provided in Fig. S3.† The instrumental method was found to have acceptable linearity ($R^2 > 0.95$) using a 4-level internal standard calibration (Table S4†) and with a linear range from 0.4 to 75.0 μg L^{-1} (Table S5†). Heavier octa-chlorinated

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compounds displayed a smaller linear range, over an order of magnitude of 10 compared to 50–75 for TCDD/Fs to HpCDD/Fs. High concentrations of PCDD/Fs are not expected in samples due to the level of regulation imposed by most governments, with even developing countries such as South Africa regulating emissions of PCDD/Fs to 0.1 ng Nm^{-3} International Toxic Equivalent (I-TEQ) and an emission license is required to operate certain facilities which could emit PCDD/Fs as promulgated in South African Department of Environmental Affairs Air Quality Act (No. 39 of 2004) (SA DEA).[31]

The technique developed here uses DMSO as a clean-up step which has a boiling point of 189 °C.[32] Consequently, GC split and septum purge lines should be routinely cleaned when using this technique due to DMSO condensation. Faster degradation of the inlet liner and injector column were also encountered compared to using more volatile injection solvents such as *n*-hexane, however these negative effects of DMSO as injection solvent were only observed after 30 to 50 injections. The PCDD/F peak shapes were found to be Gaussian and were thus not negatively affected by using DMSO.

3.2 Comparison of extraction efficiency of PCDD/Fs employing PLE and the novel end-over-end tumbling method using different solvents

PLE has been used for routine extraction of PCDD/Fs from Amberlite XAD-2 sorbent and thus served to establish a baseline reference for extraction efficiency. Similar results were found to those reported in other studies employing PLE.[33] Table 1 clearly shows that when employing end-over-end tumbling, all extraction solvents provided acceptable overall average recoveries of >70% and <130%.[20] However, extraction with *n*-hexane : acetone (1 : 1) and toluene resulted in some individual compounds being below the acceptance level with similar overall average recoveries. End-over-end tumbling employing *n*-hexane provided similar recoveries to the PLE baseline study. Notable improvement for TCDD/Fs and PeCDD/Fs was found compared to the extraction efficiency using *n*-hexane Soxhlet extraction with recovery of 54% by Kiguchi *et al.* (2006) compared to 95% achieved with *n*-hexane end-over-end tumbling.[34] The use of toluene as extraction solvent in any environmental laboratory which also tests for volatile organic compounds (VOCs) is typically avoided due to potential cross contamination, thus this solvent was excluded as an option in method development.

Toluene and acetone have polarity indices of 2.3 and 5.4, respectively, which could be the reason why the extraction recoveries were lower compared to when only *n*-hexane was used as extraction solvent for the non-polar PCDD/Fs.[35] Agglomeration of Amberlite XAD-2 sorbent was observed in the *n*-hexane and toluene extraction solvents, but this was not observed with *n*-hexane : acetone (1 : 1), where the Amberlite XAD-2 sorbent was free flowing, as acetone and water are miscible.[32] This observation was important as it was expected to negatively impact extraction repeatability, as moisture and agglomeration of the sorbent when processing real samples will limit the access of solvent to the Amberlite XAD-2 sorbent and may reduce recoveries of target analytes. The *n*-hexane end-over-end tumbling extraction was repeated to investigate this finding further and a poor average recovery of 64% was achieved compared to the previous 89%, indeed indicating a repeatability problem. These findings were also encountered by Kiguchi *et al.* (2006), when two CRMs were analysed for PCDD/Fs using an *n*-hexane Soxhlet extraction which achieved 50% and 79% recovery respectively.[34]

Consequently, an acetone : *n*-hexane (1 : 9) solvent was used based on the success of the *n*-hexane extraction efficiency and

Table 1 Extraction recoveries of PCDD/Fs obtained by PLE and end-over-end tumbling using various solvents. Acceptable recoveries were >70% and <130%. All values are reported as %

	PLE	End-over-end tumbling extraction		
PCDD/F	*n*-Hexane : acetone (1 : 1)	*n*-Hexane	*n*-Hexane : acetone (1 : 1)	Toluene
2,3,7,8-TCDF	94	95	80	82
2,3,7,8-TCDD	86	89	72	79
1,2,3,7,8-PeCDF	91	100	77	81
2,3,4,7,8-PeCDF	87	98	77	76
1,2,3,7,8-PeCDD	92	92	75	79
1,2,3,4,7,8-HxCDF	85	91	75	73
1,2,3,6,7,8-HxCDF	90	94	76	76
2,3,4,6,7,8-HxCDF	80	94	71	**60**
1,2,3,4,7,8-HxCDD	85	80	71	76
1,2,3,6,7,8-HxCDD	89	95	78	77
1,2,3,7,8,9-HxCDD	100	90	80	82
1,2,3,7,8,9-HxCDF	85	87	73	**59**
1,2,3,4,6,7,8-HpCDF	96	87	76	77
1,2,3,4,6,7,8-HpCDD	93	82	70	73
1,2,3,4,7,8,9-HpCDF	91	78	73	**67**
1,2,3,4,6,7,8,9-OCDD	99	81	67	68
1,2,3,4,6,7,8,9-OCDF	99	77	79	81
Average recovery	**91**	**89**	**75**	**74**

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Table 2 Extraction efficiency of PCDD/Fs from Amberlite XAD-2 sorbent using end-over-end tumbling and acetone : *n*-hexane (1 : 9). Acceptable recoveries were >70% and <130%. All values are in %

PCDD/F	Repeat 1	Repeat 2
2,3,7,8-TCDF	81	87
2,3,7,8-TCDD	77	83
1,2,3,7,8-PeCDF	81	88
2,3,4,7,8-PeCDF	78	84
1,2,3,7,8-PeCDD	79	85
1,2,3,4,7,8-HxCDF	77	83
1,2,3,6,7,8-HxCDF	79	82
2,3,4,6,7,8-HxCDF	70	**69**
1,2,3,4,7,8-HxCDD	75	83
1,2,3,6,7,8-HxCDD	74	82
1,2,3,7,8,9-HxCDD	80	87
1,2,3,7,8,9-HxCDF	72	69
1,2,3,4,6,7,8-HpCDF	80	87
1,2,3,4,6,7,8-HpCDD	73	78
1,2,3,4,7,8,9-HpCDF	**69**	75
1,2,3,4,6,7,8,9-OCDD	70	75
1,2,3,4,6,7,8,9-OCDF	77	88
Average recovery	76	81

the observation that acetone limited sorbent agglomeration, and the results are presented in Table 2. The extraction was repeated and acceptable average recoveries of 76 and 81% respectively were achieved. However, there were individual PCDFs in both extractions just below the acceptable 70% recovery limit which could be attributed to calibration or other analytical errors, although this is still acceptable according to US EPA Method 23. The use of mixed solvents, specifically acetone : *n*-hexane (1 : 9) in this case, did provide good results. Acetone potentially disrupts strong hydrogen bonding with the PCDD/Fs and Amberlite XAD-2 active sites improving repeatability and preventing the observed agglomeration.[34] The proposed extraction could be a potential alternative to PLE and Soxhlet based on these findings.

3.3 Partitioning of PCDD/Fs into DMSO using different volumes of *n*-hexane

From Table 3 it is evident that as the *n*-hexane volume increased, the extraction efficiency decreased. This is likely due to the lipophilic nature of PCDD/Fs. The lipophilic solubility is stronger than the electrostatic complex formed between the polarized sulphur atom of DMSO and the π-

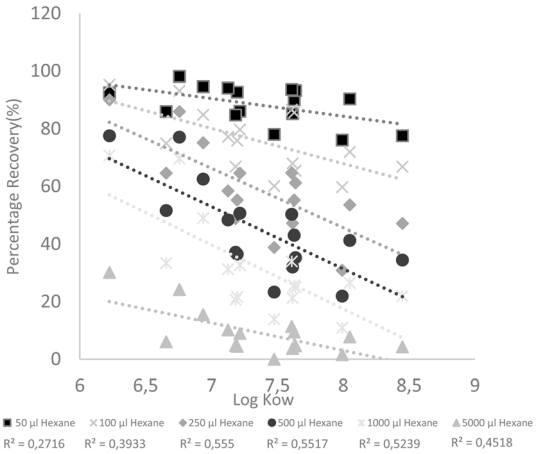

Fig. 2 Decreasing percentage recovery trend as *n*-hexane volume and log K_{ow} coefficients increase.

Table 3 Percentage recovery of PCDD/Fs partitioning into DMSO from variable *n*-hexane volumes. Acceptable recoveries were >70% and <130%. All values are in %

	DMSO volume (μL)	500	500	500	500	500	500
	n-Hexane volume (μL)	50	100	250	500	1000	5000
PCDD/F	Log K_{ow}[36]	Recovery: acceptable 70–130 (%)					
2,3,7,8-TCDF	6.225	93	95	90	78	71	30
2,3,7,8-TCDD	6.656	86	75	65	52	33	6
1,2,3,7,8-PeCDF	N/A	91	81	63	49	31	8
2,3,4,7,8-PeCDF	6.757	98	93	86	77	70	24
1,2,3,7,8-PeCDD	7.215	86	80	65	51	33	9
1,2,3,4,7,8-HxCDF	7.184	85	67	49	37	21	5
1,2,3,6,7,8-HxCDF	7.196	93	76	55	37	22	4
2,3,4,6,7,8-HxCDF	6.937	95	85	75	63	49	15
1,2,3,4,7,8-HxCDD	7.628	90	86	55	43	26	9
1,2,3,6,7,8-HxCDD	7.639	93	65	61	35	25	5
1,2,3,7,8,9-HxCDD	7.61	94	87	65	50	34	11
1,2,3,7,8,9-HxCDF	7.125	94	77	58	48	31	10
1,2,3,4,6,7,8-HpCDF	7.477	78	60	39	23	14	0
1,2,3,4,6,7,8-HpCDD	8.051	90	72	54	41	27	8
1,2,3,4,7,8,9-HpCDF	7.616	85	68	47	32	21	4
1,2,3,4,6,7,8,9-OCDD	8.45	78	67	47	34	22	4
1,2,3,4,6,7,8,9-OCDF	7.992	76	60	31	22	11	2
Average recovery		88	76	59	45	32	9

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Table 4 Repeated sample clean-up procedure employing two sequential partitioning steps using 1 : 10 *n*-hexane : DMSO. Acceptable recoveries were >70% and <130%. All values are in %

PCDD/F	Repeat 1	Repeat 2
2,3,7,8-TCDF	97	94
2,3,7,8-TCDD	90	92
1,2,3,7,8-PeCDF	98	93
2,3,4,7,8-PeCDF	99	98
1,2,3,7,8-PeCDD	95	90
1,2,3,4,7,8-HxCDF	90	82
1,2,3,6,7,8-HxCDF	97	84
2,3,4,6,7,8-HxCDF	92	90
1,2,3,4,7,8-HxCDD	86	73
1,2,3,6,7,8-HxCDD	91	91
1,2,3,7,8,9-HxCDD	90	90
1,2,3,7,8,9-HxCDF	88	88
1,2,3,4,6,7,8-HpCDF	90	91
1,2,3,4,6,7,8-HpCDD	85	85
1,2,3,4,7,8,9-HpCDF	84	90
1,2,3,4,6,7,8,9-OCDD	82	79
1,2,3,4,6,7,8,9-OCDF	73	88
Average recovery	90	88

electrons in PCDD/Fs aromatic moieties.[37,38] An initial recovery of 70–130% of PCDD/Fs was used as an acceptance window for the extractions. Fig. 2 shows a decreasing percentage recovery trend, as the $\log K_{ow}$ coefficient increases, although the R^2 is poor. This provides supporting evidence that the lipophilic interaction becomes more dominant than the partitioning of PCDD/Fs into DMSO.

A ratio of 1 : 10 *n*-hexane to DMSO was found to be the best extraction solvent ratio for PCDD/Fs based on the acceptance criteria of 70–130%, as it provided an average recovery of 88%. However, a ratio of 1 : 5 *n*-hexane to DMSO did provide an average recovery of 76% with only a few PCDD/Fs which were below the acceptance window. It should be noted that the total average recovery passes the criteria but does not leave any room for analytical deviation or error that might occur in the extraction process. Repeatability is a vital requirement of an analytical method to ensure useable data is generated, and it must be considered before a technique is routinely used.

The 1 : 10 *n*-hexane to DMSO sample clean-up procedure was therefore repeated to investigate the repeatability of the method. When the single DMSO partitioning was repeated, an average recovery of 68% was obtained which is not acceptable. A second sequential partitioning step was thus included, which yielded an average recovery of 90% and this was successfully repeated with a recovery of 88%. Additionally, all PCDD/Fs also had acceptable recoveries >70% and <130%, as shown in Table 4.

Lastly, we did encounter small peak retention time shifts on some test samples of approximately 0.012–0.035 min for ^{13}C-1,2,3,6,7,8-HxCDD, which could be due to non-polar compounds coextracted with the DMSO briefly artificially increasing the GC column film thickness with increased retention times as a result. A similar observation was made when using DMSO as a clean-up step analysing for polychlorinated biphenyls (PCBs) in transformer oil by Cromhout (2015).

3.4 Solid sample proficiency test

The optimized end-over-end tumbling extraction using 30 mL of acetone : *n*-hexane (1 : 9) and DMSO clean-up procedure was applied to the extraction of a solid proficiency test sample. Table 5 shows an average *Z*-score obtained from the PT study of −1.1 with a very good average recovery of 86% for the most toxic PCDD/Fs based on I-TEQ values (specifically for 2,3,7,8-TCDD; 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF). This is an excellent result, which proves that the novel end-over-end extraction and DMSO sample clean-up procedure is fit for purpose. Although

Table 5 *Z*-scores obtained from soil PT sample using the novel end-over-end tumbling extraction and DMSO clean-up procedure and reported values, analysed by GC-TQMS

PCDD/F	Proficiency value (pg g^{-1})	Reported value (pg g^{-1})	Recovery value (%)	Soil PT sample *Z*-score	Acceptable |*z*|
2,3,7,8-TCDF	530	515	79	−0.1	Acceptable
2,3,7,8-TCDD	459	395	86	−0.7	Acceptable
1,2,3,7,8-PeCDF	381	340	89	−0.5	Acceptable
2,3,4,7,8-PeCDF	603	551	91	−0.4	Acceptable
1,2,3,7,8-PeCDD	325	266	82	−0.9	Acceptable
1,2,3,4,7,8-HxCDF	490	374	76	−1.2	Acceptable
1,2,3,6,7,8-HxCDF	90	53	59	−2.1	Questionable – in control
2,3,4,6,7,8-HxCDF	1108	931	84	−0.8	Acceptable
1,2,3,4,7,8-HxCDD	664	497	75	−1.3	Acceptable
1,2,3,6,7,8-HxCDD	786	588	75	−1.3	Acceptable
1,2,3,7,8,9-HxCDD	615	507	82	−0.9	Acceptable
1,2,3,7,8,9-HxCDF	226	206	91	−0.4	Acceptable
1,2,3,4,6,7,8-HpCDF	220	145	66	−1.7	Acceptable
1,2,3,4,6,7,8-HpCDD	500	358	72	−1.4	Acceptable
1,2,3,4,7,8,9-HpCDF	264	208	79	−1.1	Acceptable
1,2,3,4,6,7,8,9-OCDD	1251	794	63	−1.8	Acceptable
1,2,3,4,6,7,8,9-OCDF	589	364	62	−1.9	Acceptable
Average recovery	**535**	**417**	**78**	**−1.1**	**Acceptable**

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Table 6 Recoveries of labelled PCDD surrogate standards obtained from real Amberlite XAD-2 samples, analysed by GC-TQMS

	Recovery (%)		
Labelled PCDD	Sample 1	Sample 2	Sample 3
$^{37}Cl_4$-2,3,7,8-TCDD	81	88	108
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	71	75	81
Average recovery (%)	76	82	94
Total average recovery (%)		**84**	

a slight negative bias in *Z*-scores is evident, the PT study evaluates results as acceptable if the *Z*-score is < l2l, whilst if the *Z*-score is >l2l but below l3l the result is deemed questionable but still in control. Lastly, *Z* > l3l is not acceptable. A *Z*-score of −2.1 was obtained for 1,2,3,6,7,8-HxCDF, which is deemed still in control but is not ideal. This could have been due to an analytical error in calibration or as a result of the small number of participants in this study ($N = 5$).

3.5 Recoveries from real stack emissions sampled onto Amberlite XAD-2 sorbent

Medical waste incineration is a known potential anthropogenic source of PCDD/Fs to the environment.[12–16] The developed method was thus applied to the analysis of stack emissions from such a facility located in Africa, which had been sampled onto Amberlite XAD-2. The method was adapted to cater for the elevated moisture content of the samples and to ensure low analyte concentrations could be quantified, as described in Section 2.7. Table 6 shows the recoveries achieved for the labelled surrogate PCDDs, with an overall average recovery of 84% for the three samples, proving the ability of the method to handle real sample matrix effects and to provide acceptable recoveries. The average recoveries achieved were comparable to those obtained from laboratory controlled spiking and for the solid sample proficiency testing. The native analyte concentrations in the samples are not reported due to client confidentiality, but the total PCCD/F I-TEQs in all samples were <0.1 ng Nm^{-3} I-TEQ.

4 Conclusions

The novel end-over-end tumbling technique reported here using an acetone : *n*-hexane (1 : 9) extraction solvent has been proven to be a facile extraction process which can be easily scaled up to accommodate multiple samples, which is advantageous in a routine laboratory setting. Using DMSO partitioning as a clean-up step adds to the simplicity of the technique, as no expensive prepacked solid phase extraction cartridges or column chromatography are required, nor are expensive flow management systems needed to automate the process. The reported method has been proven to be fast, repeatable, and accurate with an average PT *Z*-score of −1.1 for PCDD/Fs from a soil matrix. The method could be adapted for complex, high moisture stack emission samples and successfully extracted PCDD/Fs from stack emissions from a medical waste incinerator sampled onto Amberlite XAD-2 sorbent. This facile method is thus fit for purpose to extract PCDD/Fs from these solid matrices and shows great potential for developing country applications.

Data availability

The data supporting this research article have been included as part of the ESI.†

Author contributions

Reinardt Cromhout: conceptualization, methodology, investigation, formal analysis, writing – original draft preparation, resources and funding acquisition. Jean-François Focant: methodology, supervision, writing – review. Editing. Patricia Forbes: conceptualization, methodology, supervision writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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