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Application of low-temperature CP-Sil 88 column for the isomeric analysis of toxic 2378-substituted PCDD/Fs in incinerator flyash and sewage sludge using a triple quadrupole GC–MS/MS

Jude A. Onwudili[a,*], Yaghoub Hajizadeh[a], Safari Zainal[a], John Upton[b], Paul T. Williams[a]

[a] *Energy and Resources Research Institute, University of Leeds, LS2 9JT, UK*
[b] *Agilent Technologies (Formerly Varian), Oxford OX5 1QU, UK*

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ABSTRACT

The seventeen 2378-substituted polychlorinated dibenzo-p-dioxins and dibenzo-p-furans (PCDD/Fs) congeners have been separated and analyzed in sewage sludge and incinerator flyash samples using a CP-Sil 88 column (50 m × 0.25 mm I.D., 0.25 μm film thickness) operating at a maximum oven temperature of 240 °C. The column was used on a Varian 450-GC with a Varian 320-MS Triple Quadrupole. Calibration standards were used to determine the transition chemistries of the 2378-substituted PCDD/F congeners in the gas chromatography/mass spectrometry (GC–MS/MS) system. The five-point calibration curve for each of the congeners showed very good linearity with R^2 values greater than 0.999. The recovery of labelled compounds ranged from 50% to 120%. Analytical results from a reference flyash (BCR-490) and a reference sewage sludge (BCR-677) compared very well with the certified values, giving percentage deviations in I-TEQ (international toxic equivalents) of 4.93% and 0.53%, respectively. Results from 'real' flyash samples underscored the level of progress made in the abatement of dioxin emissions from incinerators; the old incinerator flyash contained much higher PCDD/F concentrations than the modern one. In addition, the concentrations profiles of PCDD/Fs in the 'real' sewage sludge from two UK wastewater treatment plants (WWTPs) showed that one contained a total PCDD/Fs content of 314 ng I-TEQ kg^{-1}, while the other gave a total of 53 ng I-TEQ kg^{-1}. Over an 18-month period of operation, no significant loss of analytical performance was observed from the low-temperature column.

1. Introduction

Incineration of the organic fraction of municipal and industrial solid wastes and other organic-rich wastes (e.g. sewage sludge) has the advantage of efficient energy recovery from these wastes, and also leads to dramatic mass and volume reductions. Incineration has therefore become the main alternative to the landfilling of these types of wastes. Incineration of organic wastes will continue to contribute to the achievements of the targets of the EU Landfill Directive, which requires member states to drastically reduce the amount of biodegradable wastes sent to landfills [1]. However, operation of incinerators can often generate significant negative public perception with respect to the possible release or emission of incomplete combustion products to the environment. Such emissions include the highly toxic polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF). Other sources of PCDD/Fs in the environment include releases from automobile exhausts, pulp and paper mill effluents, household washing machine effluents and the production/use of organochlorine compounds [2–4].

PCDD/Fs are a chemical group of 210 chlorinated tricyclic aromatic hydrocarbons and environmentally stable micro-pollutants, known as one of the most hazardous compounds to human health and the environment [5–7]. There are serious concerns about the high toxicity of PCDD/Fs congeners with chlorination in the 2378 positions as confirmed by studies carried out by several multi-lateral bodies such as the United States Environmental Protection Agency (USEPA), Ontario Ministry of Environment and the European Commission [8,9]. Hence in recent years, stringent regulations have been enforced to reduce the release of these and many other hazardous pollutants into the environment [10].

The required analysis of PCDD/Fs within often complex matrices poses a significant problem to the analyst. Analysis of PCDD/Fs in environmental matrices requires several steps that include extraction/isolation, clean-up and/or pre-concentration before

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[*] Corresponding author at: School of Process, Environmental and Materials Engineering, Faculty of Engineering, University of Leeds, LS2 9JT, UK.
Tel.: +44 113 343 2353; fax: +44 113 246 7310.
E-mail address: j.a.onwudili@leeds.ac.uk (J.A. Onwudili).

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measurement by gas chromatography. The high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) is the instrumental benchmark for the analyses of PCDD/Fs. However, HRGC/HRMS is very expensive and requires very skilled analysts to deliver certifiable results. Other less-costly techniques that benefit from high signal to noise ratio, may provide a cost-effective and fit-for-purpose alternative to traditional HRGC/HRMS analyses of PCDD/Fs [11]. Although, GC–MS/MS systems would result in loss of high resolution during analysis of PCDD/F in complex matrices, they can be reliably used for screening of clean non-complex matrices without the high cost associated with HRGC/HRMS systems.

The Ministry of Environment, Ontario, Canada, was the first major research group to publish the successful determination of PCDD/Fs with a gas chromatography–mass spectrometry/mass spectrometry system (GC–MS/MS) [9,12]. The authors reported that in some matrices, fewer chemical interferences were observed with GC–MS/MS than with HRGC/HRMS. Since then, the improvement in both selectivity and sensitivity of GC–MS/MS to PCDD/Fs screening has become well accepted within the scientific community [11,13].

In mass analyzers such as the HRMS, triple quadrupole and ion trap, during PCDD/F analysis various isomers, congeners and dioxin-like compounds could fragments into ions of identical mass. These interferent species could give rise to significant errors during analyte measurements. The analytical principles of the various mass analyzers are well documented. For instance, the capability of GC–MS/MS systems for PCDD/F analyses could be explained using the triple quadrupole systems as example. In the triple quadrupole MS/MS system, several ions are formed from the specific analyte in the ion source but only those selected as precursor ions, based on the developed transition chemistries of the analyte, are allowed to travel through the first quadrupole, Q1. By this filtering exercise, the precursor ions travelling through Q1 are, for example, the m/z 322 ion from 2378-TCDD and any m/z 322 ions that are due to the matrix. On entering the second quadrupole, Q2, the precursor ions are subjected to a Collision Induced Dissociation (CID) process. This is achieved by having a collision gas, typically argon, at low pressure within Q2 and applying collision energies by means of ion acceleration between a potential difference. If the collision has sufficient energy, the precursor ion may dissociate to product ions. These can then be measured by means of the third quadrupole, Q3. If the CID energies cause both the dioxin m/z 322 and the matrix m/z 322 to dissociate, the selectivity is reliant on differences in the product ions produced and monitored. Hence, by careful selection of the precursor ion, the collision energy and the product ion or ions measured by Q3, the analysis becomes very selective towards the PCDD/F

Apart from the sample preparation procedure, the chromatographic step is a key factor in successful analysis of PCDD/Fs. Dioxin-specific columns have been developed over the years to reduce the problems of co-elution of congeners, isomers and other compounds with the PCDD/Fs of interest [14–16] Recently, Reiner [17] produced a comprehensive review of the performances of various columns employed in PCDD/Fs analyses and found that at least one case of analyte co-elution was reported on each of the columns. This is due to the large number of PCDD/F congeners and similar compounds such as PCBs, PCDEs, PBDEs and halogenated dioxins and furans [17]. High peak resolutions, stability and low column bleed appear to be the main attributes of good quality dioxin analysis as a consequence of low concentrations of analytes of interest.

The generic 5% phenyl-based columns would continue to have a dominant presence in PCDD/F analysis due to its reliability, stability and good analytical separation capacity [17]. However, the development of alternative fit-for-purpose columns may provide immense benefits in terms of shorter analysis times, excellent resolution of peaks of interest and cheaper running costs. The choice of GC column is a balance between the resolution of the PCDD/Fs congeners and the stability of the phase in relation to temperature. As an example of this, the CP-Sil 88 column from Varian is excellent for specific PCDD/Fs separations but the upper operating temperature is limited to 240 °C isothermal [18,19]. This may cause a build up of high boiling point contamination from dirty sample types as injections progress. The build-up of contaminants would affect the performance of the CP-Sil 88 column if means of back-flush or column clean-up is not incorporated. By contrast, the more generic VF-5MS column can be operated up to a temperature of 325 °C, indicating less build-up of contaminants on the column but may have lower chromatographic separation ability for PCDD/Fs than the CP-Sil 88 column.

The HRMS remains the reference method of choice for the analysis of PCDD/Fs for regulatory and litigation purposes. Relatively cheaper mass analyzers such as the ion trap and the triple quadrupole for PCDD/F screening are now acceptable since the data generated are deemed fit-for-purpose for the sake of the measurement required [20,21]. Currently, GC with MS/MS in the form of ion trap and triple quadrupole detectors have been applied for the analysis of persistent organic pollutants (POPs) in various matrices including fruits and vegetables, food, fats and oils, flyash and other environmental samples [22,23]. The underlying benefit of triple quadrupole GC–MS/MS is that it offers a high degree of selectivity, good sensitivity and a high, non-specialist, throughput capability.

The two stages of mass analysis in triple quadrupole GC–MS/MS offers a possibility of the application of selected reaction monitoring (SRM), which is arguably one of the most selective and most sensitive techniques for the confirmation and quantification of trace analytes in environmental samples, in spite of the normally high background chemical noise [20]. Hayward [11] reported that the triple quadrupole MS/MS technique was reproducible, with little reduction in sensitivity or spectral quality during the analysis of the seventeen 2378-substituted PCDD/Fs. GC–MS/MS is also thought to offer an overall gain on total analysis time by allowing simultaneous identification and quantification [20], a feature comparable to high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) technique. The initial applications involving new generation of triple quadrupole instruments dates from 2003, when the potential of the technique was investigated for the screening of dioxins and furans in fish and flyash samples [20,24,25]. The use of triple quadrupole GC–MS/MS for the screening of dioxins and furans has been approved by the United States Environmental Protection Agency (USEPA) for the Department of Water [21].

Our research group has published several papers [26,27] involving the analyses of PCDD/Fs in flyash using the classical VF-5MS (5% phenyl column; DB-5 equivalent) fitted to GC with tandem ion trap MS/MS. This paper details the application of a recently acquired triple quadrupole GC–MS/MS for the analysis of PCDD/Fs. More importantly, a low-temperature, low-bleed dioxin-specific CP-Sil 88 column has been used in this work. This column has the capacity to specifically separate the seventeen 2378-substituted PCDD/F congeners. The aim here is to benchmark the results from this equipment, based on the automated method development (AMD) protocol followed, against certified reference samples of flyash and sewage sludge. Real samples of flyash and sewage sludge were employed in an ongoing research into the development of PCDD/Fs abatement technologies. Furthermore, the concentration profiles of the seventeen 2378-subsituted PCDD/Fs four ‘real’ solid samples were measured using the procedure developed. The performance of the CP-Sil 88 column, triple quadrupole GC–MS/MS and the entire analytical method over an 18-month period was also evaluated.

2. Materials and methods

2.1. Chemical standards and organic solvents

The entire analytical procedure used was based on USEPA Method 1613 for the analysis of dioxins and furans in solid samples [28]. A set of five Method 1613 calibration standard solutions (CS1–CS5) containing the 17 toxic congeners of native PCDD/F (2378-positional substitutes at concentrations between 0.5 and 2000 ng mL^{-1}) and corresponding $^{13}C_{12}$ isotope-enriched (labelled) PCDD/F standards with a purity of ≥99% were used. The calibration standards used were the EDF-9999 range from Cambridge Isotopes Limited (CIL) Inc., MA, USA, and the certificate of analysis which includes the concentration levels can be found online [29]. The $^{13}C_{12}$ labelled standard stock solution containing 17 toxic congeners of PCDD/F at the concentration level of 100 ng mL^{-1} (except for $^{13}C_{12}$-OCDD, which was 200 ng mL^{-1}) were used to quantify the corresponding native compounds by isotope dilution method. This solution was added to the samples just before the extraction process.

Furthermore, an internal standard solution containing isotopically labelled $^{13}C_{12}$-1234-TCDD and $^{13}C_{12}$-123789-HxCDD at a concentration level of 200 ng mL^{-1} in nonane were added to the purified extract prior to GC injection as a syringe standard for the determination of recovery percentages of labelled dioxins. In addition, a dioxins precision and recovery (PAR) solution was used as part of method development. All the native PCDD/F standard solutions, the labelled PCDD/F standard solutions and internal standard solutions were purchased from LGC Standards, London, UK. All the organic solvents such as acetone, toluene, dichloromethane, n-hexane and ethyl acetate used for extraction, clean-up and glassware rinsing, were grade-certified for dioxins analysis. These solvents were purchased from Sigma–Aldrich, Ltd., UK.

2.2. Solid samples

Six solid samples were obtained and analyzed for the seventeen 2378-substituted PCDD/Fs. Two of these were the certified reference flyash (BCR-490) and reference sewage sludge (BCR-677) samples containing certified PCDD/Fs values from European Commission JRC-IRMM [30,31]. The reference samples were purchased from Sigma–Aldrich, UK, and used as received for method validation. Two 'real' sewage sludge samples were obtained from two wastewater treatment plants (WWTPs) in the UK, pulverised and employed on dry matter basis. These are labelled as Sample A and Sample B in this work. Lastly, two 'real' incinerator flyash samples were analyzed. One of the flyash samples (Old MWI) was obtained from an old incinerator built around the 1970s but was closed down in the 1990s. The second flyash sample (New MWI) was obtained from a modern incinerator built in within the last decade and is currently being operated. The 'real' flyash samples were used as received.

2.3. Experimental procedure

2.3.1. Instrumental analysis of PCDD/Fs

The analyses of the seventeen 2378-substituted PCDD/Fs were performed using a Varian 450-GC attached to a Varian 320-MS Triple Quadrupole mass spectrometer (MS/MS) analyzer. The GC–MS/MS was obtained from Varian Limited (now Agilent Technologies), Oxford, UK. The GC separation of PCDD/F was carried out using a Varian CP-Sil 88 capillary column for dioxins (50 m × 0.25 mm I.D., 0.25 μm film thickness). Ultra high purity helium was used as a carrier gas at a constant flow-rate of 1 mL min^{-1}. 2 μL samples were injected to the GC in splitless injection mode at 270 °C. The column oven temperature program was initially 140 °C held for 2 min, ramped at 45 °C min^{-1} to 190 °C with no holding time, and ramped at 5 °C min^{-1} to 240 °C held for 31.89 min giving the total runtime of 45 min. This method enabled the rapid elution of the solvent in injected sample matrices and best separation of PCDD/F throughout the column. The transfer line temperature was held at 250 °C and once the PCDD/Fs arrived at the ion volume, which was maintained at 200 °C, ionization was by EI (electron ionization). The detector was set to operate using the Extended Dynamic Range (EDR) with centroid scan position. More details of the MS conditions are shown in Table 1.

Table 1
MS conditions used in this work.

MS conditions	
Tune	EI autotune
Filament current	50 μA
Electron energy	70 eV
Source temperature	200 °C
Source pressure	18 mTorr
CID gas pressure	1.50 mTorr
Manifold pressure	7.7 μTorr
Dwell times	See Table 2

Ultra high purity argon was used for Collision Induced Dissociation (CID) gas for the dissociation of precursor ions in the collision cell at a pressure of 1.50 mTorr. The acquisition of data was carried out with a Varian MS Workstation Version 6.9.2. Data acquisition was initially delayed for 12 min, since preliminary work showed that none of the 2378-substituted PCDD/Fs eluted before this time.

2.3.2. Method validation procedure

Detection limits were estimated for each of the seventeen 2378-substituted congeners by the injection of dilute solutions of the calibration standards. Together with a good match of MS/MS transition chemistries, the minimum concentration of each congener which resulted to a signal to noise ratio of 3 or greater was regarded as the operational detection limit (ODL). However, the practical detection limit (PDL) determination was carried out only on the individual standard for the native 2378-TCDD congener. In the procedure, the 10 μg mL^{-1} standard solution of 2378-TCDD was diluted to 5 times the estimated detection limit of this congener and spiked onto 5 g of analytical sand. Eight individual spikes were made and extracted according to the procedure in Section 2.3.3. The PDL for 2378-TCDD was calculated as 3 times the standard deviation of the eight replicate samples. A method blank sample was prepared by extracting and analysing dioxin-free analytical sand.

Further, the triple quadrupole GC–MS/MS analysis of dioxins and furans was validated using the precision and recovery (PAR) standard and the reference samples. The PAR standard, which has the same concentrations of 2378-substituted PCDD/Fs as in CS4, was spiked onto 5 g of analytical sand at 10 μL and 20 μL volumes to make two concentration levels within the CS1–CS5 range in the final extracts prior to GC analysis. The analytical-grade sand was obtained from Sigma–Aldrich, UK. The two spiked sand samples were denoted as PAR10 and PAR20.

2.3.3. Sample extraction, clean-up and concentration

A modified USEPA Method 1613 was applied for the extraction of PCDD/Fs in solid matrices [31]. The reference flyash and sewage sludge samples, PAR-spiked sand samples and the MWI flyash samples were initially digested with 10 mol L^{-1} HCl, filtered, rinsed with ultra high purity water and dried in the oven at 105 °C for 1 h. Each of the dried samples was then spiked with $^{13}C_{12}$-labelled PCDD/F toxic congeners prior to extraction and left to equilibrate for 2 h by occasionally tumbling the samples. The samples were loaded into the 11 mL extraction cell, filling it with pre-cleaned dioxin grade sand and extracted using Dionex ASE-100 with toluene as the extraction solvent. Based on EPA Method 3545A, the extraction procedure of the ASE system was set for the temperature of 200 °C and pressure of 1500 psi [32,33]. The extracts were evaporated on a rotary evaporator to reduce the volume to about 100 μL. The concentrated extract was transferred to a vial, by rinsing the flask several times with aliquots of hexane to ensure quantitative transfer. The remaining toluene solvent was removed by repeated (2–3 times) blow-down of the extract and reconstituting with hexane according to Method 1613 [31]. Finally, the extract now in about 12 mL of hexane was ready for the clean-up procedure.

Clean-up of the samples from interferences was achieved using an automated Power-Prep^{TM} Fluid Management System (FMS), based on sequential use of acidic–basic multilayer silica, basic alumina and activated carbon adsorbents, pre-packed in disposable Teflon column and hermetically sealed. The default dioxin clean-up method was set up and applied for the purifying the sample extracts. This is a certified method by the company, and recovery percentage of 75–90% for different PCDD/Fs congeners have been reported in literature, using this system [34,35]. The collected fraction was evaporated at 60 °C under vacuum to reduce the volume then micro-concentrated by nitrogen blow-down at 45 °C. Finally, 10 μL nonane was added to the fraction so that the PCDD/Fs were preferentially partitioned into the nonane. The fraction was blown down to remove the remaining hexane and to achieve a final PCDD/Fs fraction of 10 μL in nonane. Prior to GC–MS/MS analysis, an additional 10 μL of CIL internal standard was added and equilibrated on an ultrasonic bath.

2.3.4. *Column maintenance*

In a low-temperature column significant build-up of high boiling point contamination might occur, leading to loss of column performance that may result in poor separation of analytes and general reproducibility of analytical results. As a precaution, periodic blank injections were carried out as part of a regime of quality assurance protocol, even when PCDD/F analyses were not planned. Furthermore, to check the integrity of the CP-Sil 88 column, full scan acquisitions have been run at preset time intervals of four weeks.

3. Results and discussion

3.1. *Method development*

For each of the 2378-substituted PCDD/Fs of interest, the experimental parameters were determined using the automatic method development (AMD) for the reaction monitoring (SRM) facility present in the Triple Quadrupole 320-MS. The result of such method development gave the full condition set for the dioxins and furans found in the CS calibration standards as shown in Table 2. The

Table 2
Established chromatographic conditions and characteristic transition chemistries for 2378-substituted PCDD/Fs with the GC-TQ/MS instrument.

No	Compound name	RT (min)	Transition 1	Transition 2	Collision energy (eV)	Dwell times (ms)	Segment
1	2378-TCDD	13.335	320 → 257	322 → 259	20	100	1
2	2378-TCDD C13	13.335	332 → 268	334 → 270	20	100	
3	2378-TCDD Cl37	13.346	328 → 263		20	100	
4	1234-TCDD C13	13.497	332 → 268	334 → 270	20	100	
		14					
5	2378-TCDF	15.672	304 → 241	306 → 243	35	75	2
6	2378-TCDF C13	15.672	316 → 252	318 → 254	35	75	
7	23478-PeCDF	15.771	338 → 275	340 → 277	30	75	
8	23478-PeCDF C13	15.771	350 → 286	352 → 288	30	75	
		16					
9	23478-PeCDD	16.573	354 → 291	356 → 293	25	100	3
10	23478-PeCDD C13	16.573	366 → 302	368 → 304	25	100	
		18					
11	123478-HxCDF	19.158	372 → 309	374 → 311	35	100	4
12	123478-HxCDF C13	19.158	384 → 320	386 → 322	35	100	
13	123678-HxCDF	19.332	372 → 309	374 → 311	35	100	
14	123678-HxCDF C13	19.332	384 → 320	386 → 322	35	100	
		19.6					
15	12378-PeCDF	20.037	338 → 275	340 → 277	30	100	5
16	12378-PeCDF C13	20.037	350 → 286	352 → 288	30	100	
		20.2					
17	123478-HxCDD	20.597	388 → 325	390 → 327	25	100	6
18	123478-HxCDD C13	20.597	400 → 336	402 → 338	25	100	
19	123678-HxCDD	20.782	388 → 325	390 → 327	25	100	
20	123678-HxCDD C13	20.782	400 → 336	402 → 338	25	100	
		21.5					
21	123789-HxCDD	22.196	388 → 325	390 → 327	25	100	7
22	123789-HxCDD C13	22.196	400 → 336	402 → 338	25	100	
		23					
23	1234678-HpCDF	23.762	408 → 345	410 → 347	30	75	8
24	1234678-HpCDF C13	23.762	420 → 356	422 → 358	30	75	
25	123789-HxCDF	24.061	372 → 309	374 → 311	35	75	
26	123789-HxCDF C13	24.061	384 → 320	386 → 322	35	75	
		25					
27	234678-HxCDF	26.368	372 → 309	374 → 311	35	100	9
28	234678-HxCDF C13	26.368	384 → 320	386 → 322	35	100	
29	1234678-HpCDD C13	28.302	436 → 372	438 → 374	25	100	
30	1234678-HpCDD	28.208	424 → 361	426 → 363	25	100	
		29					
31	1234789-HpCDF	30.114	408 → 345	410 → 347	30	100	10
32	1234789-HpCDF C13	30.114	420 → 356	422 → 358	30	100	
		32					
33	OCDF	37.963	442 → 379	444 → 381	35	100	11
34	OCDD C13	39.018	470 → 406	472 → 408	25	100	
35	OCDD	39.018	458 → 395	460 → 397	25	100	

Table 3
Variations of relative response factors (RRFs) for native 2378-substituted PCDD/Fs congeners.

RT (min)	PCDD/Fs	CS1	CS2	CS3	CS4	CS5	Mean	% RSD
13.309	2378-TCDD	1.10	1.07	1.04	1.02	1.08	1.06	2.71
13.306	2378-TCDD CL37	5.08	5.06	4.71	4.78	4.82	4.89	3.47
15.603	2378-TCDF	1.16	1.06	1.01	0.99	1.02	1.05	6.48
15.758	23478-PeCDF	1.08	1.04	0.98	0.97	0.99	1.01	4.50
16.505	12378-PeCDD	1.15	1.01	0.91	0.94	0.95	0.99	9.64
19.175	123478-HxCDF	1.02	0.99	0.95	0.91	0.95	0.96	4.30
19.352	123678-HxCDF	1.27	1.00	0.95	0.92	0.97	1.02	14.0
19.966	12378-PeCDF	1.13	1.05	1.04	1.04	1.06	1.06	3.67
20.605	123478-HxCDD	0.99	0.98	1.06	1.06	0.98	1.01	4.20
20.60	123678-HxCDD	1.01	0.96	0.88	0.97	0.86	0.93	6.63
22.15	123789-HxCDD	1.04	1.06	0.97	0.99	0.97	1.01	4.13
23.889	1234678-HpCDF	1.18	1.15	1.06	1.04	1.05	1.10	5.91
23.99	123789-HxCDF	1.13	0.97	0.94	0.91	0.90	0.97	9.44
26.266	234678-HxCDF	0.95	0.96	0.88	0.87	0.87	0.90	5.04
28.211	1234678-HpCDD	1.12	1.09	1.07	1.09	1.08	1.09	1.60
30.164	1234789-HpCDF	1.13	1.19	0.99	1.05	1.06	1.08	7.26
38.176	OCDF	1.26	1.04	0.96	1.00	1.16	1.08	11.9
39.181	OCDD	1.29	1.20	1.09	1.05	1.06	1.14	9.17

retention times were categorized into 11 segments and the corresponding scan method for each segment was introduced in order to avoid the co-elution of PCDD/Fs isomers [26]. The time segments were defined following the acquisition of full scan spectral data. In other words, the segment breaks were placed where it was possible to do so, using retention time windows. The chromatographic outlines presented in Fig. 1 shows the excellent separation of the CS1–CS5 PCDD/Fs congeners of interest on the CP-Sil 88 column.

For each congener, a $^{13}C_{12}$ labelled homologue was present as internal standard, except for 2378-T^{37}CDD and OCDF, whose $^{13}C_{12}$ homologues were not in the standard mixtures. The concentrations of these two PCDD/Fs were determined using $^{13}C_{12}$ labelled 2378-TCDD and OCDD, respectively. Once the scanning characteristics of the PCDD/Fs have been determined, the CS1–CS5 calibration standards were used to construct each of the calibration curves required for the quantification of 17 PCDD/Fs. The curves obtained exhibit high linearity, indicating very good response of the triple quadrupole GC–MS/MS throughout the concentration range as reported by Worrall et al. [25]. All the congeners gave very linear responses throughout the CS calibration range, with R^2 values of 0.999.

Table 3 presents the relative response factors (RRF) for all the PCDD/Fs in the calibration standard mixtures throughout the range. The table shows that apart from 2378-TC^{37}DD, all the analyzed congeners gave RRF values of approximately 1.00. This was expected since each congener used its $^{13}C_{12}$ as internal standard such that their transition chemistries would be near identical. The % RSD obtained are comparable to the work of Worrall et al. [25]. However, this is clearly more specific and different from the work of March et al. [2] and Worrall et al. [25], who used internal standard method with a labelled compound for all the PCDD/Fs congeners. The low % RSD in the RRF values is an indication of the stability of response of the instrument during PCDD/Fs analysis. Moreover, Worrall et al. [25] have shown that a similar triple quadrupole system gave very high operational stability in terms of response factors, ion ratios and retention times, in addition to very low limits of detection (LODs) for all the seventeen 2378-substituted PCDD/Fs in Carp extract. In this work, the operational detection limit (ODL) for the 2378-TCDD congener was 0.160 ng kg^{-1}, considering a recovery of 73.9% for its labelled analogue (shown in Table 4).

3.2. Method validation

The entire analytical procedure, including sample digestion, extraction and isolation of the PCDD/Fs from solid samples through to the instrumental analysis with the GC–MS/MS, was validated using the BCR-490 and BCR-677 reference materials and samples of PCDD/Fs-free analytical sand spiked with two different concentrations of precision and recovery standard (PAR) solution. The PAR solution contained only native PCDD/Fs but the labelled homologues were added just before extraction.

3.2.1. Initial Precision and Recovery (IPR)

The mean recoveries of the labelled congeners during the analysis of the spiked sand under IPR (Initial Precision and Recovery) tests are shown in Table 4. These were calculated based on the addition of internal standard solution, comprising $^{13}C_{12}$-1234-TCDD and $^{13}C_{12}$-123789-HxCDD, just before sample injection into the GC. The recoveries were generally higher for PAR20 compared to PAR10, for both PCDDs and PCDFs. Since the final sample volume prior to injection on to the GC–MS/MS was kept at 20 μL, the concentrations of the native compounds in the PAR10 would be half their concentrations in PAR20.

The differences in the mean recoveries between PAR10 and PAR20 could be attributed to the relative concentrations of the labelled compounds in both samples. Since the same amount of labelled compound was added to both PAR samples, the concentration ratios between each native compound and its labelled analogue would be higher in PAR10 than in PAR20. This may have resulted in the seemingly lower recoveries obtained for PAR10 due to elevated levels of labelled compounds. However, general recoveries as high

Table 4
Recoveries of the labelled 2378-PCDD/Fs congeners from IPR experiments.

PCDD/F compounds	Recovery of labelled compounds (%)	
	PAR10	PAR20
Dioxins		
2378-TCDD C13	73.9	83.3
12378-PeCDD C13	76.7	81.6
123478-HxCDD C13	50.0	66.1
123678-HxCDD C13	59.4	70.5
1234678-HpCDD C13	68.2	99.1
OCDD C13	69.6	94.8
Furans		
2378-TCDF C13	66.2	77.3
12378-PeCDF C13	70.0	71.6
23478-PeCDF C13	72.6	81.2
123478-HxCDF C13	57.2	77.6
123678-HxCDF C13	59.4	83.1
234678-HxCDF C13	65.1	98.9
123789-HxCDF C13	71.5	103
1234678-Hp7CDF C13	67.3	118
1234789-HpCDF C13	73.5	120

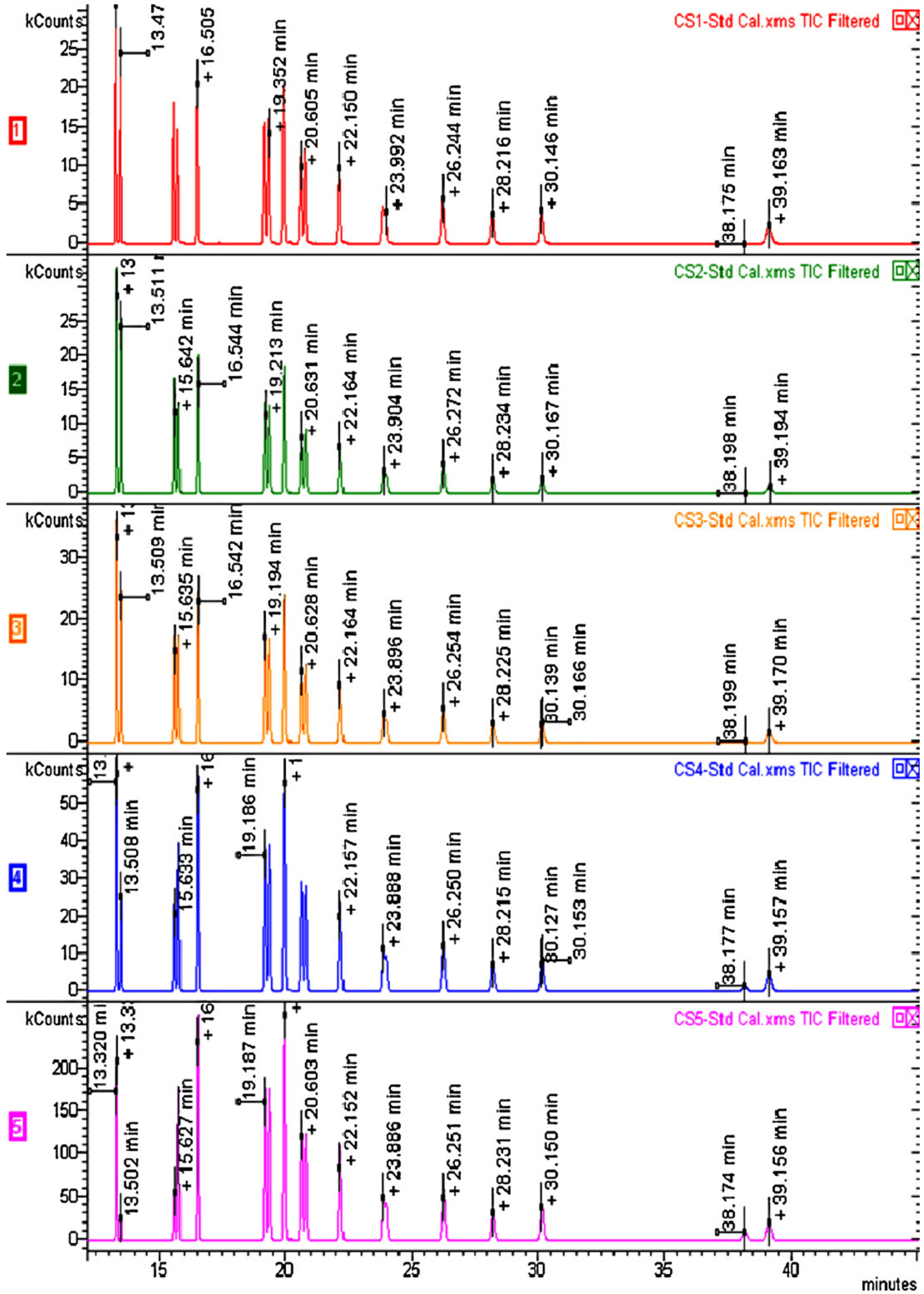

Fig. 1. Chromatographic outlines of CS1–CS5 calibration standards on the CP-Sil 88 column.

as 50% and above can be regarded as acceptable in dioxin analyses [26].

3.2.2. *Analysis of reference samples*

The results from the analysis of the reference flyash and reference sewage sludge are shown in Figs. 2 and 3, respectively. Duplicate analyses of the samples were performed and results compared with the certified values. The results presented are the concentrations of 2378-substituted PCDD/Fs. It is significant that the results of 2378-TCDF with the GC–MS/MS showed good agreement to the certified value, indicating acceptable separation of analytes on the CP-Sil 88 column, as well as a good overall analytical procedure. The Often other isomers of 2378-TCDF, such as the 1249-, 1279-, 2346-, 2347- and 2348-TCDF, are co-eluted on a DB-5 column [36]. The % SD for all the congeners of interest was 4.93 for the flyash and 0.53 for the sewage sludge, in terms of I-TEQ. The higher % SD for the reference flyash was probably due to the relatively higher concentrations of PCDD/Fs in this sample

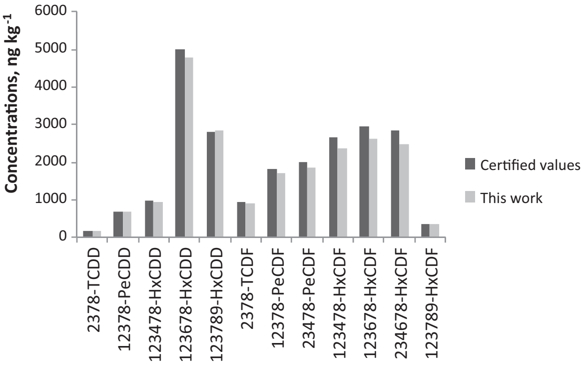

Fig. 2. Comparison between analytical results and certified values in BCR-490 flyash.

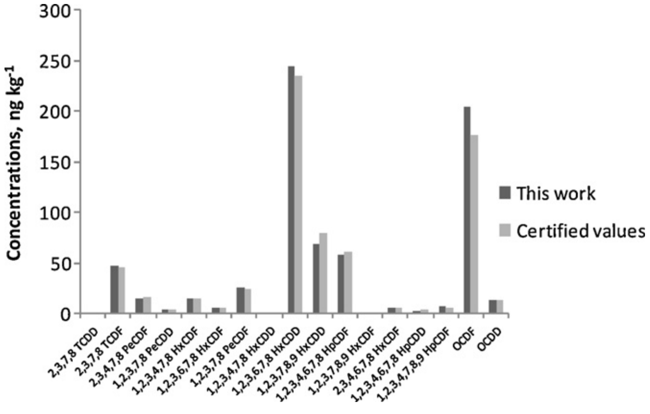

Fig. 3. Comparison between analytical results and certified values in BCR-677 sewage sludge.

compared with the reference sewage sludge sample. These results showed very good agreement between the experimental values and the certified values, considering that the certified values were based mainly on analysis by HRMS, using the conventional dioxins capillary columns [37].

However, other PCDD/Fs with similar transition chemistries were detected in the chromatograms. For example, the analysis of incinerator flyash, showed the presence of several other PCDD/Fs with the same transitions as those defined specifically for the 2378-substituted homologues. These may be oxygenated and/or positional isomers of the seventeen congeners. In these cases, retention times and RRF values may be used to identify and quantify them.

3.3. *Analysis of 'real' solid samples*

3.3.1. *Flyash samples from two incineration plants*

The developed analytical procedure was used for the screening the concentration profiles of all seventeen 2378-substituted PCDD/Fs in two real-world flyash samples obtained from incineration plants. Results obtained are presented in Table 5. The old incineration plant was constructed in the 1970s when the restriction of dioxin emissions was practically a non-issue; hence such incinerators were constructed without any specific requirement for dioxin abatement. The dioxin content in ng kg^{-1} of the old plant flyash was nearly 20 times that of the modern plant. Similarly, in terms of total toxicity equivalents (ng TEQ kg^{-1}), the dioxin content in the flyash from the modern plant was over 33 times lower compared to the old plant. The trends obtained in this work are in agreement with the work of Cunliffe and Williams [26], who employed tandem gas chromatography with ion trap analyzer for the similar samples from different incinerators. The general trends in the results appear similar even though the previous work [26] was carried out with the classical VF-5MS column. This shows that the CP-Sil 88 column can be applied for the analysis of real samples. These values indicate that significant reduction of dioxin emission has been achieved over the course of three or more decades due to the deployment of PCDD/F mitigation technologies including better operation of incinerators, the use of PCDD/F traps such as scrubbers and activated carbon.

3.3.2. *Sewage sludge samples*

Table 6 shows the results of duplicate analyses of two sewage samples from waste water treatment plants (WWTPs) in the UK. The total PCDD/Fs in WWTP A was about 14,900 ng kg^{-1}

Table 5
Concentration profiles of 2378-substituted PCDD/Fs from municipal waste incineration (MWI) flyash samples.

PCDD/Fs in MWI flyash	Old MWI (ng kg^{-1})	Old MWI (ng TEQ kg^{-1})	Modern MWI (ng kg^{-1})	Modern MWI (ng TEQ kg^{-1})
Dioxins				
2378-TCDD	1830	1830	21	21
12378-PeCDD	6300	3150	221	111
123478-HxCDD	7210	720	186	18.6
123678-HxCDD	34,800	3480	194	19.4
123789-HxCDD	19,400	1940	563	56.3
1234678-HpCDD	ND	ND	4180	41.8
OCDD	ND	ND	6180	6.18
$\sum$PCDD	69,540	11,120	11,545	273
Furans				
2378-TCDF	3300	330	248	24.8
12378-PeCDF	8150	408	323	16.1
23478-PeCDF	2400	1200	299	149
123478-HxCDF	13,400	1340	337	33.7
123678-HxCDF	11,930	1190	326	32.6
123789-HxCDF	5380	538	93	9.30
234678-HxCDF	ND	ND	429	42.9
1234678-HpCDF	185,000	1850	1520	15.2
1234789-HpCDF	ND	ND	293	2.93
OCDF	ND	ND	ND	ND
$\sum$PCDF	229,600	6860	3870	327
$\sum$PCDD/F	299,140	17,980	15,420	874

Table 6
Concentrations of 2378-substituted PCDD/Fs from WWTP sewage sludge samples.

PCDD/Fs in sewage sludge	WWTP A		WWTP B	
	ng kg^{-1}	ng TEQ kg^{-1}	ng kg^{-1}	ng TEQ kg^{-1}
Dioxins				
2,3,7,8 TCDD	9.83	9.83	26.0	26.0
1,2,3,7,8 PeCDD	29.3	14.6	0.00	0.00
1,2,3,4,7,8 HxCDD	86.4	8.64	3.80	0.38
1,2,3,6,7,8 HxCDD	85.5	8.55	16.2	1.62
1,2,3,7,8,9 HxCDD	36.4	3.64	5.77	0.58
1,2,3,4,6,7,8 HpCDD	893	8.93	1220	12.2
OCDD	317	3.17	57.3	0.06
ΣPCDD	1460	57	1330	41
Furans				
2,3,7,8 TCDF	206	20.6	22.3	2.23
2,3,4,7,8 PeCDF	466	23.3	71.1	3.55
1,2,3,4,7,8 HxCDF	227	22.7	4.93	0.49
1,2,3,6,7,8 HxCDF	214	21.4	9.76	0.98
1,2,3,7,8 PeCDF	165	82.6	4.56	2.28
1,2,3,4,6,7,8 HpCDF	1710	17.1	284	2.84
1,2,3,7,8,9 HxCDF	64.9	6.49	ND	–
2,3,4,6,7,8 HxCDF	529	52.9	ND	–
1,2,3,4,7,8,9 HpCDF	268	2.68	ND	–
OCDF	6760	6.76	ND	–
ΣPCDF	10,610	257	397	12.4
ΣPCDD/F	14,920	314	1730	53.2

compared to WWTP B with 1730 ng kg^{-1}, both on dry matter basis (d.m.). These correspond to PCDD/Fs concentrations of 314 ng I-TEQ kg^{-1} (d.m.) compared to 53 ng I-TEQ kg^{-1} (d.m.), for the WWTP A and WWTP B samples, respectively. Therefore, the I-TEQ concentration of the PCDD/Fs was about 6 times higher in the WWTP A than in the WWTP B. The reported concentration range for the 2378-substituted congeners for sewage sludge in the UK was between 9 ng I-TEQ kg^{-1} to 206 ng I-TEQ kg^{-1} [38]. Stevens et al. [39] reported that in UK sewage sludges, the homologue group pattern of the PCDD/Fs was dominated by the HpCDD and OCDD. According to these authors, such dominance of highly chlorinated homologues was consistent with other sewage sludges and there was no correlation between the degree of industrial input and the PCDD/F concentrations in their study. Their results tend to agree with the isomer profiles obtained in WWTP B, where the 2378-substituted HpCDD and OCDD dominate. However, in WWTP A samples, the 2378-substituted congeners of OCDF and OCDD dominate the profiles; along with HpCDF, HpCDD HxCDF and PeCDF. It is not however clear why the samples from WWTP A contained appreciably high concentrations of PCDD/Fs. One plausible reason could be that the WWTP A plant received and treated more chlorinated effluents than WWTP B.

3.4. Stability and repeatability of analytical system

One of the apparent challenges of a low-temperature column for PCDD/Fs analyses is the possible build up of higher boiling-point contaminants, which could lead to significant loss of column performance. Therefore, the stability and repeatability of the analytical system (including the column and MS/MS) was tested by the injection of the CS3 calibration standard (mid-range concentrations). This was performed six months apart. Several injections in batches were performed in intervals of one week in each case. Table 7 shows the mean-batch concentrations of three injections of CS3 PCDD/F congeners over the 18-month period. Results indicate very low and acceptable % RSD values which in turn show significant stability of response as well as very good repeatability

Table 7
Repeatability tests with CS3 calibration standard.

S/N	PCDD/F	Nominal CS3 conc. (ng mL^{-1})	02/11/09 (ng mL^{-1})	05/07/10 (ng mL^{-1})	31/01/11 (ng mL^{-1})	% RSD[a]
1	2378-TCDD	10	10.3	9.86	10.1	2.20
2	2378-TCDD CL37	10	9.84	9.65	9.7	0.98
3	2378-TCDF	10	9.99	9.65	9.8	1.70
4	23478-PeCDF	50	48.6	47.9	49.7	1.81
5	12378-PeCDD	50	49.9	48.4	47.6	2.34
6	123478-HxCDF	50	49.9	50.6	51.9	2.03
7	123678-HxCDF	50	47.8	49.3	50.5	2.71
8	12378-PeCDF	50	48.7	49.9	49.1	1.22
9	123478-HxCDD	50	52.8	50.1	51.5	2.70
10	123678-HxCDD	50	50.9	51.2	49.1	2.27
11	123789-HxCDD	50	52.5	52.6	49	4.10
12	1234678-HpCDF	50	54.1	50.7	52.6	3.41
13	123789-HxCDF	50	52.1	51.9	52.8	0.95
14	234678-HxCDF	50	50.6	50.7	50.4	0.31
15	1234678-HpCDD	50	53.6	48	50.1	5.66
16	1234789-HpCDF	50	49.5	49.9	51.1	1.67
17	OCDF	100	116	110	113	3.32
18	OCDD	100	104	105	102	1.53

[a] Calculated from nominal concentration.

of injection and analytical separations. The consistency of the injection results shows that the low-temperature CP-Sil 88 column was stable over this period. This level of performance is appreciable and suggests that the column was suitable for the analyses of the seventeen 2378-substituted dioxins and furans, without any significant loss of column integrity.

4. Conclusion

A method development procedure has been undertaken for the measurement of isomeric profiles of 2378-substituted PCDD/Fs using a GC fitted with a triple quadrupole mass spectrometer (MS/MS). The sample clean-up procedure was carried out with an FMS Power-Prep^{TM} instrument, which showed good capability for removing interferences. The column used was a CP-Sil 88 column, operated at a maximum temperature of 240 °C. The column showed good separation of the PCDD/Fs of interest and no significant build-up of high boiling-point contaminants in the column has been observed so far for over a year. The transition chemistries of the compounds of interest were determined for both native and labelled homologues. The analytical equipment showed very good stability and linearity with injections of calibration standards. The analytical procedure was validated by the use of method blank, initial precision and recovery (IPR) as well as reference flyash and sewage sludge samples. Recoveries of labelled compounds ranged from 50% to 120%, while the native compounds gave similar recovery percentages or better. Analysis of real environmental samples gave results as expected. The procedure developed was very good and fit for purpose for the determination of PCDD/Fs in solid matrices such as flyash and sewage sludge samples. Over an 18-month period, the low-temperature column did not show any significant loss of performance.

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