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Establishing an alternative method for the quantitative analysis of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans by comprehensive two dimensional gas chromatography–time-of-flight mass spectrometry for developing countries

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ABSTRACT

Comprehensive Gas Chromatography–Time-of-Flight Mass Spectrometry (GC × GC–TOFMS) methodology has been refined for the analysis of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in samples with different matrices. This is specifically for application in developing countries where access to gas chromatography–high resolution mass spectrometry (GC–HRMS) and highly skilled personnel is limited. The method, using an Rxi-5 Sil MS column in the first dimension (^{1}D) coupled with an Rtx-200 column in the second dimension (^{2}D), was used to quantify PCDDs and PCDFs in different environmental sample matrices. The results were compared with those obtained using GC–HRMS and good agreement was observed. The limit of detection (LOD) for the method (300 fg on column for spiked soil samples) was determined using an Rxi-XLB (^{1}D) column coupled with an Rtx-200 column (^{2}D). Preliminary South African sample results are also discussed. Isomer specificity for different tetrachloro dibenzo-*p*-dioxins (TCDDs) and tetrachloro dibenzofurans (TCDFs) was investigated using a commercial standard. Adequate resolution was achieved. The method as described has great attraction for developing countries being both financially and operationally favourable.

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1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) constitute two classes of structurally related chlorinated aromatic hydrocarbons that are both highly toxic and produced as by-products during a variety of chemical and combustion processes [1]. Due to their hydrophobic character and resistance to metabolic degradation these substances exist as complex congener mixtures in the environment.

South Africa has no established gas chromatography–high resolution mass spectrometry (GC–HRMS) facility for dioxin analysis [2]. This situation is not as a result of a lack of need; indeed in developing countries there is probably a greater need for strict control of effluent and waste disposal [3], but results from a shortage of finance available for the purchase of specialized scientific equipment, and also a shortage of skilled personnel needed for the optimum use and maintenance of such technology. The need for environmental monitoring is essential to ensure sustainable growth without exposing the population to dangerous toxins [4].

The lack of GC–HRMS facilities in South Africa (and other developing countries) mandates the development of an alternative solution that is affordable, easy to manage and aligned to the country's needs. The resultant analytical approach must be robust, must provide fast turnaround times and must accurately screen for many classes of environmental pollutants in a single analysis. This alternative methodology is not intended as a replacement for existing, accepted technology for PCDD and PCDF analysis, but rather as a rapid screen, so that only positive samples can be sent for expensive GC–HRMS analysis in qualified overseas laboratories.

The National Metrology Institute of South Africa (NMISA), in collaboration with various institutions, launched the project towards a comprehensive screening method for multiple classes of persistent organic pollutants (POPs) in one run using Comprehensive Gas Chromatography–Time-of-Flight Mass Spectrometry (GC × GC–TOFMS) [5,6]. There are numerous challenges that have to be overcome with this approach. Chief amongst these being the separation of the PCDD/F congeners from each other and from matrix interference and the sensitivity considerations to allow accurate quantitative measurement down to 500 fg for 2,3,7,8-

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TCDD as required by US EPA Method 1613 [7]. The ultimate goal of this methodology would be a comprehensive screening method for multiple classes of POPs in one run [5,6] coupled with accurate quantitation of important pollutant classes. This paper focuses on PCDDs and PCDFs and describes the analysis of the seventeen priority PCDD/F congeners mandated by EPA Method 1613 [7].

GC × GC–TOFMS was considered the preferred technique as it provides both the selectivity (added peak capacity of GCxGC) [1,8] and the sensitivity (focusing effect of the modulator) [9] needed for this demanding environmental analysis. It is expected that the selectivity lost due to low resolution mass spectrometry (vs HRMS) will be offset by the higher selectivity of the extra gas chromatographic dimension. Moreover, as full range mass spectral data are always acquired (detailed full range mass spectral acquisition for selected classes of POPs is described in a previous paper [6]), it is not a target compound technique such as single ion monitoring (SIM), and is ideal for screening for multiple classes of environmental pollutants in a single analysis [1,10–14]. Retrospective data mining of archived data will be possible when future focus turns to new target compounds, e.g., the brominated dioxins.

This paper describes results on the analysis of PCDD/Fs aimed at establishing a dioxin capability for South Africa. More specifically, optimization of the column combinations and temperature programming are reported and results evaluated against sensitivity and isomer specificity guidelines of the US EPA Method 1613 [7].

2. Experimental

2.1.1. Chemicals

US EPA Method 1613 calibration and verification solutions (EPA-1613CVS), labelled calibration solutions (EPA-1613LCS), internal standard spiking solution (EPA-1613ISS) and cleanup standard stock solution (EPA-1613CSS) were chosen for spiking and calibration purposes. These solutions were purchased from Wellington Laboratories (Guelph, Canada) and contained the seventeen native and corresponding mass-labelled PCDD/F congeners in nonane. The isomer specificity and window defining standard (EDF-4147 *GC Retention Time Window Defining Solution and Isomer Specificity Test Standard*) was purchased from Cambridge Isotope Laboratories (MA, USA).

All solvents (hexane, methylene chloride, nonane and iso-octane) were high purity grade from Burdick and Jackson (Honeywell International Inc., USA). Sodium sulphate (Na_2SO_4) anhydrous, sodium chloride (NaCl) and potassium hydroxide (KOH) were from Sigma–Aldrich (Chemie GmbH, Germany). Liquid nitrogen and chromatographic pure grade helium gas (99.999%) were purchased from Air Products (Kempton Park, South Africa). The packing material used for sample extraction recovery was Chem Tube Hydromatrix® from Varian Inc. (supplied via SMM Instruments, South Africa). The consumables for the recovery determinations using the *Total Rapid Preparation* (TRP) system (Fluid Management Systems, Watertown, MA, USA) included a 3 g "in cell" cleanup column (packed with Na_2SO_4, and Hydromatrix®) for pressurized liquid extraction. Further sample cleanup included high capacity PCB-free Silica (#PCB HCDS), acid base neutral Silica (#PCBS: ABN), Alumina (#PCBA: BAS) and Carbon (#PCBC: CCG).

2.1.2. Sample preparation

Sediment samples were provided by the Environmental Protection Agency (EPA) in Taiwan. Extraction, clean-up and GC–HRMS analysis of these samples were performed by the same laboratory. The sample extraction procedure for the PCDD/Fs follows the US EPA Method1613 [7]. The samples were analysed using both GC–HRMS (according to US EPA Method 1613) and GC × GC–TOFMS (Table 1c, supporting information).

Soil and sediment samples were also taken from selected sites within South Africa. The soil and sediment samples were extracted and underwent clean-up procedures adapted from existing US EPA Methods (3545 [15]; 3620 C [16]; 3600 C [17] and 1613 [7]) at the North West University (NWU), Potchefstroom, South Africa.

The samples were transported and stored at 4 °C, air-dried, homogenized and sieved (0.5 mm). After mixing 40 g of soil with an equal amount of Na_2SO_4 and spiking with 10 μl of $^{13}C_{12}$ labelled internal standard (100 ng/ml, EPA-1613CSL), the samples were extracted with a mixture of high-purity hexane and dichloromethane (DCM) using accelerated solvent extraction (ASE). Prior to clean-up, the extracts were spiked with 10 μl of EPA-1613CSS clean-up standard ($^{37}Cl_4$-2,3,7,8-TCDD). The exact clean-up procedure depended on the target compound. All extracts underwent gel permeation chromatography (GPC) to remove all molecules outside the size range of the target compounds. After GPC the sample was evaporated to 0.5 ml, reconstituted to 1.5 ml (iso-octane) and then split into separate portions (0.5 ml) for three different clean-up procedures.

The dioxin clean-up procedure consisted of a concentrated sulphuric acid wash, followed by a 5% NaCl wash (60 min), a KOH wash (10–15 min) and lastly a second 5% NaCl wash (60 min). Samples were filtered through pre-extracted glass wool covered with Na_2SO_4 to remove residual water and evaporated to 0.5 ml in iso-octane.

The recovery determinations were performed using the *Total Rapid Preparation* (TRP) system [11,18]. Two 10 g sterilized soil samples were spiked with 10 μl of $^{13}C_{12}$ labelled internal standards (100 ng/ml) and extracted using the TRP *Pressurized Liquid Extraction* module [18], solvent evaporated using the C*oncentrator* module, labelled clean-up standard (10 μl) added (EPA-1613CSS) and clean-up using the TRP *Power Prep* module with final solvent evaporation to 0.5 ml [18,19]. The extraction and analytical method followed US EPA Method 1613 [7].

2.1.3. GC × GC–TOFMS analysis

The GC × GC–TOFMS system used in this study at the NMISA was a Pegasus 4D (LECO Corporation, St Joseph, MI, USA). The system has an Agilent 7890 GC equipped with an Agilent 7683B autosampler, a secondary oven and a dual stage modulator. Liquid nitrogen (LN_2) was used for the cold jets and synthetic air for the hot jets. The LN_2 levels were maintained using an AMI Model 186 liquid level controller. One set of analyses was performed on the system at the EPA (Taiwan) laboratory using the *Restek* Rxi-5silms/Rtx-200 column combination (similar to column combination 2, Table 1). This system is identical to the system at the NMISA where the remainder of the analyses was performed.

Three specific GC column combinations were evaluated during this study. These columns included an Rtx-Dioxin 2/Rtx-PCB, an Rxi-5 Sil MS/Rtx-200 and an Rxi-XLB/Rtx-200 (Table 1a, supporting information). All the columns used were obtained from *Restek* (Bellefonte, PA, USA). Primary and secondary columns were connected using a press-tight connector.

The system was tuned on the 414 ion from the conventional perfluorotributylamine (PFTBA) mass calibrant. This is different from the standard tuning procedure and is an attempt to improve the signal intensity at the higher mass range [21]. All instrument functions and data processing were managed with the *LECO ChromaTOF* software (version 4.24). Manual review of all peak identifications and integrations was performed using this software. Library searching was performed using a PCDD/F *user* library compiled from the PCDD/F standards.

Table 1
GC × GC–TOFMS method parameters for Rxi-XLB column set.

First dimension column	Rxi-XLB (30 m × 0.25 mm id × 0.25 μm df)
Second dimension column	Rtx-200 (2.0 m × 0.18 mm id × 0.20 μm df)
Carrier gas	Helium
Injection mode	Splitless
Injection volume	2 μl
Solvent	Iso-octane
Flow mode	Constant flow
Flow rate	1.0 ml min^{-1}
Inlet purge time	60 s
Inlet purge flow	20 ml min^{-1}
Inlet total flow	21 ml min^{-1}
Inlet temperature	250 °C
Oven equilibration time	0.5 min
^{1}D column temperatures	80 °C for 1 min, ramp at 20 °C min^{-1} to 220 °C, no hold, at 2 °C min^{-1} to 240 °C, no hold, at 1 °C min^{-1} to 250 °C, no hold, at 5 °C min^{-1} to 260 °C, no hold, at 1 °C min^{-1} to 270 °C, no hold, at 5 °C min^{-1} to 310 °C, hold for 2 min
^{2}D column temperatures	100 °C for 1 min, ramp at 20 °C min^{-1} to 240 °C, no hold, at 2 °C min^{-1} to 260 °C, no hold, at 1 °C min^{-1} to 270 °C, no hold, at 5 °C min^{-1} to 280 °C, no hold, at 1 °C min^{-1} to 290 °C, no hold, at 5 °C min^{-1} to 330 °C, hold for 2 min
Transfer line temperature	270 °C
Modulator temperature offset	30 °C
Modulation period	4 s
Hot pulse time	1.0 s
Cool time between stages	1.0 s
Acquisition delay	600 s
Start mass	100 amu
End mass	520 amu
Acquisition rate	50 spectra s^{-1}
Detector voltage	1950 V
Electron energy	−70 V
Mass defect setting	−40 mu/100 u
Ion source temperature	250 °C

The Rxi-5 Sil MS/Rtx-200 column combination was selected for quantitation of the Taiwan results and the Rxi-XLB/Rtx-200 column combination was selected for final quantitation of the South African spikes and sediment sample results (see Section 3). All instrument parameters are shown in Table 1 and in the supporting information (Table 1a–d).

3. Results and discussion

3.1. Selectivity using different column combinations

Selectivity starts with good chromatography. The choice of column combinations for comprehensive GC × GC–TOFMS has been detailed in the literature [11–20]. The first approach for dioxin analysis at the NMISA was based on the method by Hoh [21]. A 60 m Rtx-Dioxin2 column was used (Table 1a and b, supporting information) and the total run time for the analysis was longer than 60 min. After analysis of the data, it was evident that considerable wrap-around of the dioxin compounds (hepta and octa) had occurred [22], as can be seen from Fig. 1. This could have an impact on quantitation as broader peaks have a detrimental effect on area determination at the low levels (500 fg for 2,3,7,8-TCDD) stipulated in US EPA Method 1613 [7], and it is also possible that target compounds can wrap-around into areas of high matrix contamination.

This led to the decision to consider a 30 m column and to standardize on a conventional stationary phase such as a 5% diphenyldimethylsiloxane; a columns that most South African laboratories would have available. Thus the Rxi-5 Sil MS/Rtx-200 column combination (Table 1a and c, supporting information) was initially selected to optimize the dioxin method using GC × GC–TOFMS. This column combination was also used at the EPA in Taiwan by one of the authors to analyse the fly ash and sediment samples by GC × GC–TOFMS for comparison with GC–HRMS data from the same institution. Good separation of the seventeen priority PCDD/F congeners was achieved (Fig. 2) with this combination, and the method was used to calculate the results detailed in Table 2. Since the total toxic equivalency (TEQ) value is combined from the individual toxic equivalency factors (TEFs) for all seventeen PCDD/F congeners [23], the contribution of each congener to the total value has to be established and it is therefore essential to separate the seventeen PCDD/F congeners from each other, especially the seven hexachlorodibenzo-*p*-dioxins and furans (HxCDD/Fs). By using the excellent efficiency, inertness, low bleed and high thermal stability of the low polarity *Restek* Rxi-XLB column phase in the first dimension coupled with the mid-polarity phase Rtx-200 (cross-bond trifluoropropylmethyl polysiloxane) in the second dimension, this separation was achieved (see Fig. 3). This provided more confidence

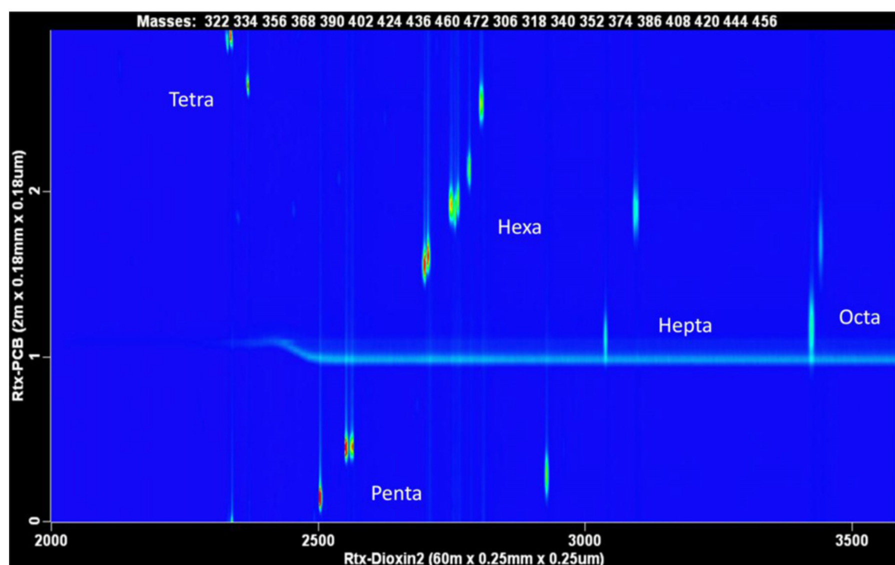

Fig. 1. ^{2}D selected ion contour plot for the 17 priority PCDD/Fs using the method by Hoh [21] showing significant wrap around [9]. Possible problems that can be encountered with wrap around are peak broadening of the later eluting compounds and interference from column bleed and matrix. The $^{13}C_{12}$-1,2,3,4-TCDD labelled compound accounts for the 18 peaks that are visible.

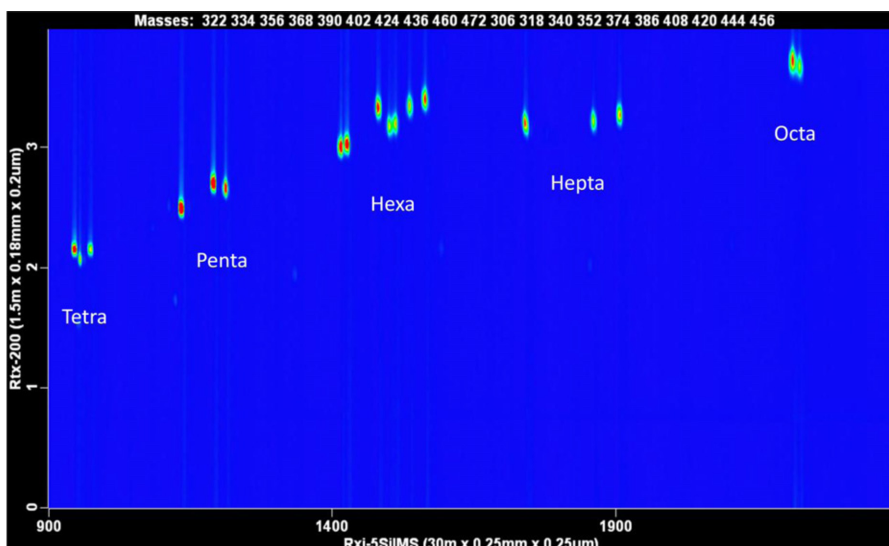

Fig. 2. ^{2}D selected ion contour plot for the 17 priority PCDD/Fs using the Rxi-5 Sil MS/Rtx-200 column combination (including additional 1,2,3,4-$^{13}C_{12}$-TCDD). The PCDD/Fs are well resolved from each other. The method is optimized for the separation of all the priority pollutants not just the dioxin compounds and matrix interference, so the entire ^{2}D chromatographic space has not been utilised.

that the final calculated result reflects the individual contributions of the seventeen priority PCDD/Fs.

The GC multi-step temperature program and MS method (Table 1) was developed with the goal in mind of separating and quantifying, in a single analysis, the many classes of POPs that might possibly be present in a real sample. This strategy was aimed at utilising the added selectivity of GC × GC and the full range mass spectra generated by TOFMS for separating and identifying components even at low levels.

The first dimension separation (Fig. 8) demonstrates the isomer selectivity in conventional ^{1}D dioxin analysis using the XLB phase However, the ^{1}D separation using GC–TOFMS cannot reach the levels required in US EPA Method 1613. In addition, in environmental samples, both matrix interference and any column bleed can interfere with the detection and quantitation of target analytes. As an example of this argument, Fig. 4 shows that the second dimension separation is essential to pull the target PCDD/F analytes away from matrix interference. Plotting the selected ions for 2,3,7,8-TCDF/2,3,7,8-[$^{13}C_{12}$]-TCDF (m/z = 306, 318) and 2,3,7,8-TCDD/2,3,7,8-[$^{13}C_{12}$]-TCDD (m/z = 322, 334) indicate the presence in the sample of other more abundant compounds containing ions which would interfere in the analysis. Even with the interference that is present, the target peaks are well resolved and can easily be quantified, providing the added selectivity and sensitivity needed to reach the low levels at which these PCDD/F compounds occur in environmental samples. Fig. 4 also highlights the need for good sample clean-up, as inadequate sample clean-up could conceivably produce unacceptable peak overlap.

3.2. Sensitivity, quantitation and limits of detection

The results for the US EPA Method 1613 [7] and US EPA Method 8290a [24] standard set can be used as an indication of the limits of detection (LOD) possible using this methodology, bearing in mind that, in dioxin work, the achievable LOD depends critically on the matrix interference, and so will vary from sample to sample.

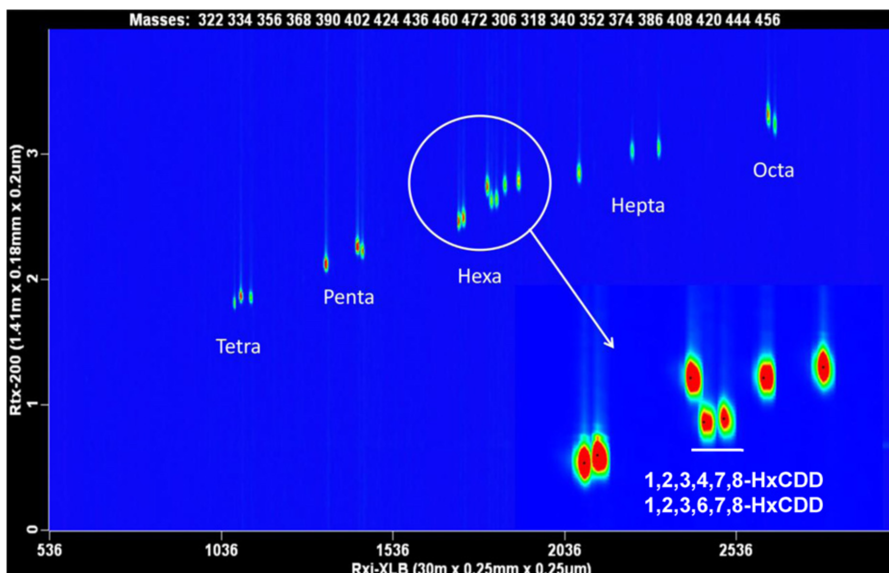

Fig. 3. ^{2}D selected ion contour plot for the 17 priority PCDD/Fs using the Rxi-XLB/Rtx-200 column combination. The PCDD/Fs are again well resolved, especially the 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD isomers.

Table 2
GC–HRMS and GC × GC–TOFMS sediment sample results.

	Sample 1 (fly ash)				Sample 2 (sediment)				Sample 3 (sediment)				Sample 4 (standard, no extraction)			
	TOF[a]	HRMS[b]	TOF TEF[c]	HRMS TEF[d]	TOF[a]	HRMS[b]	TOF TEF[c]	HRMS TEF[d]	TOF[a]	HRMS[b]	TOF TEF[c]	HRMS TEF[d]	TOF[a]	HRMS[b]	TOF TEF[c]	HRMS TEF[d]
2,3,7,8-TCDD	3.2	2.6	16	13	68	73	65	69	55	50	268	242	19	18	19	18
1,2,3,7,8-PeCDD	14	16	69	78	4.3	4.8	4.1	4.5	5.3	9.3	26	45	75	77	75	77
1,2,3,4,7,8-HxCDD	14	13	6.8	6.6	6.5	7.6	0.6	0.7	3.9	5.0	1.9	2.4	49	52	4.9	5.2
1,2,3,6,7,8-HxCDD	17	16	8.4	8.0	14	14	1.3	1.3	17	13	8.1	6.4	51	56	5.1	5.6
1,2,3,7,8,9-HxCDD	12	9.4	6.0	4.7	10	11	1.0	1.0	11	9.2	5.2	4.8	54	41	5.4	4.1
1,2,3,4,6,7,8-HpCDD	123	77	6.1	3.8	197	124	1.9	1.1	140	85	6.8	4.1	69	35	0.7	0.4
OCDD	283	240	0.1	0.1	1000	940	0.1	0.1	724	703	0.3	0.3	96	100	0.01	0.01
2,3,7,8-TCDF	25	21	12	11	47	47	4.4	4.5	10	18	4.9	8.6	37	42	3.7	4.2
1,2,3,7,8-PeCDF	43	46	11	11	20	14	0.9	0.8	7.8	6.3	1.9	1.5	50	48	2.5	2.4
2,3,4,7,8-PeCDF	107	129	270	320	215	246	102	116	19	14	46	33	51	49	25	25
1,2,3,4,7,8-HxCDF	93	100	46	48	217	235	20	22	131	118	63	57	50	51	5.0	5.2
1,2,3,6,7,8-HxCDF	105	105	52	52	21	17	2.0	1.6	24	21	12	10	50	45	5.0	4.5
2,3,4,6,7,8-HxCDF	197	174	98	86	20	16	1.9	1.5	12	6.1	5.9	3.0	50	44	5.0	4.4
1,2,3,7,8,9-HxCDF	32	29	16	14	8.1	6.7	0.8	0.6	9.7	8.0	4.7	3.9	72	66	7.2	6.6
1,2,3,4,6,7,8-HpCDF	400	412	20	21	670	740	6.3	7.0	466	490	23	24	47	48	0.5	0.5
1,2,3,4,7,8,9-HpCDF	58	48	2.9	2.4	17	13	0.2	0.1	33	29	1.6	1.4	52	43	0.5	0.4
OCDF	232	266	0.1	0.1	850	920	0.1	0.1	1390	1400	0.7	0.7	97	103	0.01	0.01
TOTAL PCDD/Fs			640	680			210	230			480	450			165	162

HR–GCMS results provided courtesy of Dr. Peng, EPA, Taiwan (see acknowledgements), using the 1998 World Health Organization (WHO) Toxicity Equivalency Factor (TEF) values.
[a] TOF: GC × GC–TOFMS results (pg/μl).
[b] HRMS: GC–HRMS results (pg/μl).
[c] TOF TEF: GC × GC–TOFMS TEQ results calculated per weight of sample extracted (ng WHO TEQ kg^{-1}).
[d] HRMS TEF: GC–HRMS TEQ results (ng WHO TEQ kg^{-1}).

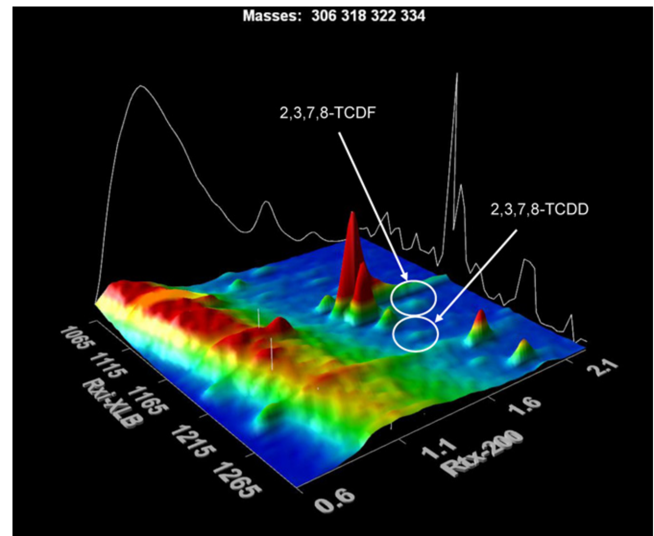

Fig. 4. Surface plot showing the position of the 2,3,7,8-TCDF and 2,3,7,8-TCDD in the presence of matrix interference. Fig. 3 has shown the separation of standards and the position of the TCDD/F isomers in the chromatographic space. In this chromatogram it is evident that the TCDD/F peaks would not be detected in a ^{1}D separation; the peaks would be buried under the matrix interference. The ^{2}D separation clearly removes the target analytes from the matrix interference, allowing for accurate quantitation.

In terms of toxicity [1,3,4,23,25,26], and US EPA Method 1613 [7] requirements, the crucial low level compound under evaluation is the 2,3,7,8-TCDD. Using the EPA-1613CVS standard calibration set described in Section 2 (0.5–200 pg/μl), a calibration curve was constructed for the seventeen components of the standard set. For the 2,3,7,8-TCDD the calibration curve obtained was linear with a correlation coefficient of 0.99972 (slope and intercept: $+0.0102161x + 0.000851118$) and an average response factor (aveRF) of 1.061. The ability of the method to reach the required low level quantitation was then further investigated.

The low level standard (CS1) which contains 2,3,7,8-TCDD at 0.5 pg/μl was used to determine the LOD possible for this compound. Using the method described in EPA Method 1613, the signal/noise (S/N) ratio for the ion of m/z 322 for 2,3,7,8-TCDD was calculated [24] as 20 (EPA Method 1613 requires this ratio to be >10 [7]). This confirms that the method can reach the LOD required by EPA Method 1613. The relevant chromatographic peaks used for the quantitation of the 2,3,7,8-TCDD standard at a concentration of 0.5 pg/μl, together with the corresponding labelled standard at a concentration of 100 pg/μl are shown in Fig. 5 (Fig. 5a and b, supporting information). Even for the lowest level standard (0.5 pg/μl) the chromatographic peak for the ion at 322 atomic mass units (amu) was easily discernible and could be accurately quantified. The mass spectra obtained for the 2,3,7,8-TCDD/F are easily searched against a user library, with a similarity match of 77% for 500 pg on column (Fig. 5c and d, supporting information).

To confirm that this LOD was attainable in soil samples, two 10 g sterilized soil samples were spiked with native dioxins at concentration levels of 500 pg (sample 1) and 2000 pg (sample 2) for 2,3,7,8-TCDD. After adding labelled material, extraction (ASE), clean-up (GPC) and concentration (NWU, Potchefstroom, South Africa, *see acknowledgements*), it was possible to calculate LODs for 2,3,7,8-TCDD of 322 fg for sample 1 and 353 fg for sample 2. These calculations were made by determining the S/N for the ion of m/z 322 and extrapolating linearly to a S/N of 3:1. These results are consistent with the LOD determination using the low level standard detailed above, and provide assurance that the method has the sensitivity for low level dioxin determination. Quantitation was

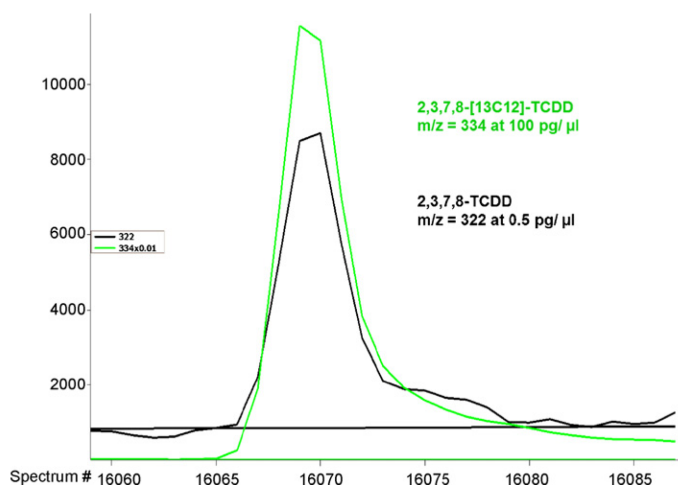

Fig. 5. Extracted ion profiles for the native (m/z = 322) and labelled (m/z = 334) 2,3,7,8-TCDD showing the sensitivity that can be achieved for the lowest standard (0.5 pg/μl), meeting US EPA Method 1613 criteria [7]. In order to compare these two concentration levels, the labelled peak was reduced to scale.

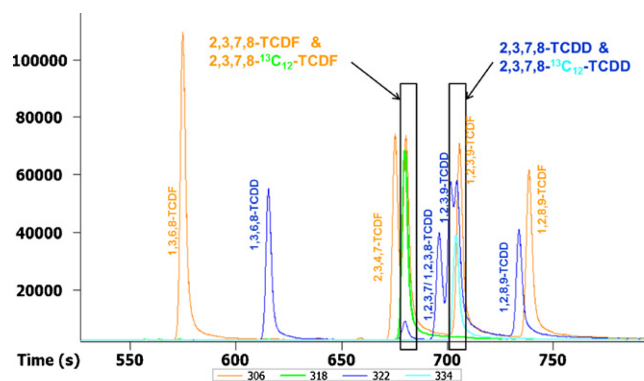

Fig. 6. ^{1}D separation on the Rxi-5 Sil MS column using the CIL EDF-4147 test mix to demonstrate isomer specificity for the GC columns employed for the determination of PCDD/F congeners. The ^{1}D GC separation followed the temperature programming parameters given in the certificate of analysis. There is incomplete separation for the 2,3,4,7- and 2,3,7,8-TCDF isomer peaks and chromatographic overlap for the 1,2,3,7/1,2,3,8/1,2,3,9- and 2,3,7,8-TCDD isomer peaks.

performed by measuring peak area ratios (native/labelled material) and then using either the calibration curve or the relative response factor (RRF), where RRF = Area ^{12}C/Area ^{13}C × [^{13}C]/[^{12}C]. Here [^{12}C] and [^{13}C] are the concentrations of native and labelled material, respectively. This RRF value is a measure of the different response obtained for the native and labelled components. The value is then used to calculate the concentration of unknown material in the sample to be quantified. During the calculation a correction for a blank (BC) can be made if required; [^{12}C] = ((Area ^{12}C/Area ^{13}C x [^{13}C]/RRF) – BC) × (1/sample weight), the result given as ng kg^{-1} [7].

The *ChromaTOF* software tool can be used package provides options to calculate the RRF from the calibration table. Compound purity can also be checked using ion ratios values; comparison of the expected ion ratio with the calculated ion ratio is an indication of the purity of the compounds quantified and the validity of the calibration (Table 3).

3.3. Isomer specificity

For reliable dioxin analysis, US EPA Method 1613 stipulates that the beginning and ending retention times for the dioxin and furan isomers be defined and isomer specificity for the GC columns employed for the determination of 2,3,7,8-TCDD/F must be demonstrated [7]. It is not necessary to monitor the window-defining compounds if only 2,3,7,8-TCDD and 2,3,7,8-TCDF are to be determined. For our purposes, an isomer-specificity test standard (CIL EDF-4147, *Cerilliant*), containing the most closely eluted isomers separated on a 5%:95% diphenyl:dimethylpolysiloxane column phase, was selected to further validate the column choice for dioxin analysis using GC × GC–TOFMS. This standard was chosen as ^{1}D separation is crucial for the success of the analysis. The column performance of the XLB phase is similar to a 5%:95% diphenyl:dimethylpolysiloxane phase for dioxin analysis (Figs. 2 and 3) so it is reasonable to assume that this standard would provide a good test for the XLB phase too.

The GC parameters, as stipulated in the certificate for the CIL EDF-4147 (*GC Retention Time Window Defining Solution and Isomer Specificity Test Standard*), were followed in order to mimic the US EPA Method 1613 separation as closely as possible (Table 1d, supporting information). This would then serve as the point of departure for further evaluating the NMISA GC parameters with regard to isomer specificity. The TCDD and TCDF compounds present in the EDF-4147 test mix will be discussed as this separation is paramount for the set of applications included in this study.

The ^{1}D [22] separation obtained using the standard CIL conditions is shown in Fig. 6. Chromatographic overlap is evident of the 2,3,7,8-TCDD/2,3,7,8-[$^{13}C_{12}$]-TCDD compounds by other TCDD isomers and by a TCDF isomer. The four dioxin isomer peaks (1,2,3,7/1,2,3,8-, 1,2,3,9- and 2,3,7,8-TCDD) co-elute to such a degree that it would not be possible to accurately quantify 2,3,7,8-TCDD with one dimensional GC.

The ^{1}D GC separation was carried out using the same Rxi-5 Sil MS column, but using the NMISA GC ^{1}D method parameters (Table 1), is shown in Fig. 7. As with the previous results, the co-elution with 2,3,7,8-TCDD is still apparent, meaning that accurate quantitation of this compound would not be possible.

However, using the Rxi-XLB column phase, the ^{1}D separation of the 2,3,7,8-TCDF/2,3,7,8-[$^{13}C_{12}$]-TCDF pair and the 2,3,7,8-TCDD/2,3,7,8-[$^{13}C_{12}$]-T pair from the other isomers contained in the standard mix was achieved (Fig. 8). A new overlap is evident, but is resolved using ^{2}D and the peak *deconvolution* software (Note Fig. 10: the two black dots indicating peak apex separation via deconvolution for isomer peaks 1,2,3,7/1,2,3,8- and 1,2,3,9-TCDD).

Table 3
Purity Ion Ratio Check: Calibration table results for 2,3,7,8-TCDD.

Area (TeCDD)	Cert Conc. (TeCDD)	Calc. Conc.	% Diff. Conc.	Ion Ratio % Diff.	Calc. Ion Ratio	Expected Ion Ratio	Ratio	RF
ND	0.10	NC	NC	NC	0.000	NC	NC	NC
840	0.25	0.29	14	8.07	0.454	0.494	0.003	1.277
1552	0.50	0.54	7	8.33	0.453	0.494	0.006	1.198
5893	2.00	2.02	1	1.24	0.500	0.494	0.023	1.126
27947	10.00	9.76	2	9.18	0.539	0.494	0.109	1.089
106259	40.00	34.97	13	10.4	0.546	0.494	0.390	0.975
575557	200.00	184.33	8	7.44	0.531	0.494	2.060	1.028

ND: not detected; NC: not calculated.

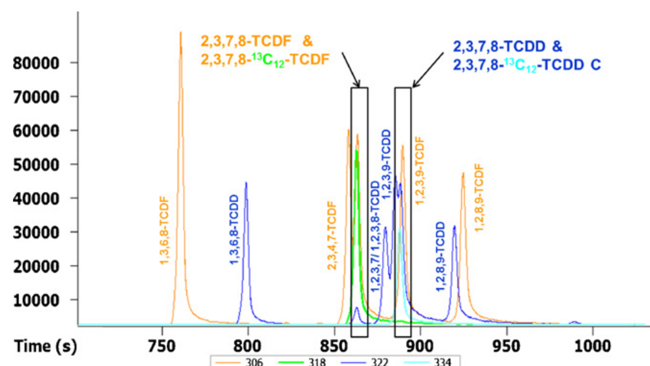

Fig. 7. Isomer specificity ^{1}D separation using the same Rxi-5 Sil MS column. A multi-step temperature program was used (Table 1). There is not much difference in chromatographic separation (compared with Fig. 6), but it does confirm compliance with the US EPA Method 1613 requirements for using a DB-5 equivalent phase.

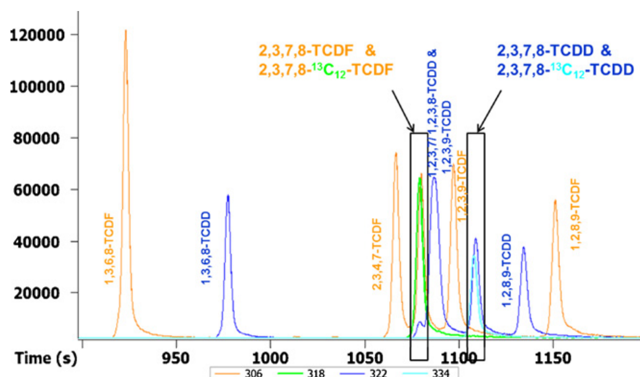

Fig. 8. Isomer specificity ^{1}D separation on the Rxi-XLB column using the multi-step temperature program (Table 1). There is definite improvement with the phase change between the Rxi-5 Sil MS and XLB columns. The 2,3,7,8-TCDF/2,3,7,8-[$^{13}C_{12}$]-TCDF and the 2,3,7,8-TCDD/2,3,7,8-[$^{13}C_{12}$]-TCDD isomers are well separated from the other isomers.

Moreover, the overlap of compounds not necessary for quantitation by EPA Method 1613 is of no importance for final TEQ determination.

The separation in ^{2}D for the Rxi-5 Sil MS/Rtx-200 and Rxi-XLB/Rtx-200 column combinations can be seen in Figs. 9 and 10. From the chromatograms (^{2}D contour plots) it is evident that the 2,3,7,8-TCDF/2,3,7,8-[$^{13}C_{12}$]-TCDF and the 2,3,7,8-TCDD/2,3,7,8-[$^{13}C_{12}$]-TCDD separation with the XLB phase has been achieved with no overlapping interference from the other TCDD/F isomers present.

3.4. Sample analysis

The analysis of dioxins based on standards is necessary in order to familiarize the laboratory analyst with these toxic compounds and their intrinsic chromatographic properties. Method development and optimization has to be followed by method validation. The laboratory of the EPA in Taiwan is equipped with both GC–HRMS and GC × GC–TOFMS systems and it was possible to compare the results provided from a set of samples analysed by the standard GC–HRMS method with the ones we obtained using their GC × GC–TOFMS. Table 2 shows an example of the results for two sediment samples, one fly ash sample and one check standard. There is good agreement between the GC–HRMS and GC × GC–TOFMS data sets, indicating that the GC × GC–TOFMS method is capable of handling this complex dioxin analysis for environmental samples.

With the TRP system, it was possible to establish recovery data for two blank samples spiked at different concentration levels. The results shown in Table 4 provided a set of data that corroborated the calculated versus actual results. The toxicity of the various compounds is defined in terms of the toxic equivalency factor (TEF) [23]. The 2,3,7,8-TCDD is assigned a value of one, and the other dioxin and furan compounds are compared to it. Thus 2,3,7,8-TCDF is rated one tenth as toxic as 2,3,7,8-TCDD [26]. When reporting the results, these values are then added together to give a total toxicity equivalent for the seventeen dioxins and furans. As can be seen in Table 4, the calculated value obtained was 280 ng TEQ/kg and the actual analytical result obtained was 290 ng TEQ/kg, with recoveries between 70 and 110%. These results confirm that the GC × GC–TOFMS method meets the US EPA 1613 criteria.

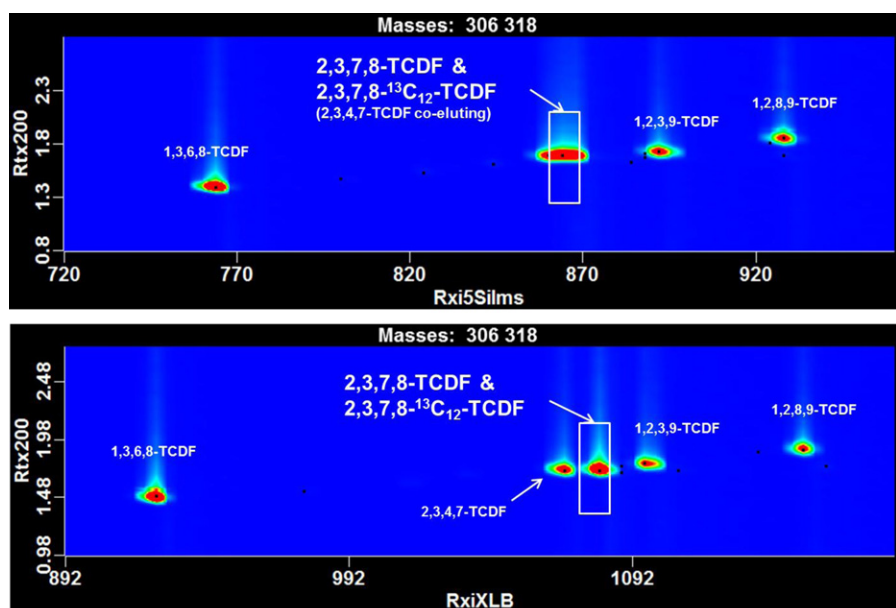

Fig. 9. Isomer specificity ^{2}D TCDF separation using the Rxi-5 Sil MS/Rtx-200 and the Rxi-XLB/Rtx-200 column combinations. The black dots are deconvoluted peak apexes for the TCDF isomers. 2,3,4,7-TCDF co-elutes with 2,3,7,8-TCDF/2,3,7,8-[$^{13}C_{12}$]-TCDF with the Rxi-5 Sil MS column phase. These peaks are well separated using the XLB column phase.

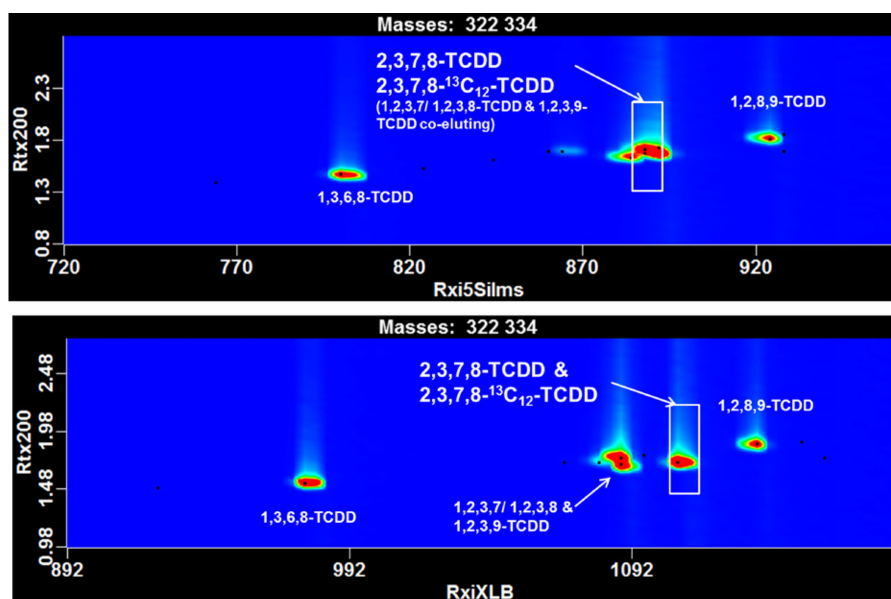

Fig. 10. Isomer specificity ²D TCDD separation using the Rxi5Silms/Rtx-200 and the Rxi-XLB/Rtx-200 column combinations. 1,2,3,7/1,2,3,8 and 1,2,3,9-TCDD co-elute with 2,3,7,8-TCDD/2,3,7,8-[$^{13}C_{12}$]-TCDD with the Rxi-5 Sil MS column phase. Note the position of the 2,3,7,8-TCDF and 1,2,3,9-TCDF peaks. With the XLB phase, the elution order has shifted. The dioxin isomer co-elution of 1,2,3,7/1,2,3,8 and 1,2,3,9-TCDD with 2,3,7,8-TCDD is resolved, clearly separating the 2,3,7,8-TCDD/2,3,7,8-[$^{13}C_{12}$]-TCDD peaks. 1,2,3,7/1,2,3,8-TCDD and 1,2,3,9-TCDD are resolved in ²D.

Table 4
South African GC × GC–TOFMS soil spike recovery results (Rxi-XLB/Rtx-200 column combination).

Compound	¹D RT	²D RT	[a]TOF	[b]Spike	[c]TOF TEF	[d]Spike TEF	% Recovery
2,3,7,8-TCDD	1116	1.76	29	25	29	25	114
1,2,3,7,8-PeCDD	1436	2.12	124	127	124	127	98
1,2,3,4,7,8-HxCDD	1812	2.50	108	127	11	13	85
1,2,3,6,7,8-HxCDD	1824	2.52	128	127	13	13	100
1,2,3,7,8,9-HxCDD	1848	2.62	90	127	10	12	70
1,2,3,4,6,7,8-HpCDD	2216	2.90	129	127	1.3	1.3	102
OCDD	2616	3.16	195	254	0.1	0.1	77
2,3,7,8-TCDF	1088	1.76	26	25	2.5	2.5	101
1,2,3,7,8-PeCDF	1332	2.02	92	127	2.7	3.8	72
2,3,4,7,8-PeCDF	1420	2.16	103	127	30	40	81
1,2,3,4,7,8-HxCDF	1720	2.34	118	127	12	13	93
1,2,3,6,7,8-HxCDF	1732	2.38	131	127	13	13	103
2,3,4,6,7,8-HxCDF	1800	2.62	140	127	14	13	110
1,2,3,7,8,9-HxCDF	1888	2.68	140	127	14	13	110
1,2,3,4,6,7,8-HpCDF	2064	2.72	120	127	1.2	1.3	95
1,2,3,4,7,8,9-HpCDF	2296	2.92	95	127	1.0	1.3	75
OCDF	2636	3.08	176	254	0.1	0.1	69
TOTAL PCDD/Fs					280	290	

2005 World Health Organization (WHO) Toxicity Equivalency Factor (TEF) values [23].
[a] TOF: GC × GC–TOFMS results (ng kg^{-1}).
[b] Spike: calculated (ng kg^{-1}).
[c] TOF TEF: GC × GC–TOFMS TEQ results (ng WHO TEQ kg^{-1}).
[d] Spike TEF: calculated (ng WHO TEQ kg^{-1}).

The collaborative project with North West University (NWU), Potchefstroom, South Africa, consists of numerous soil and sediment samples taken from various strategic sites in South Africa. The samples contain various POPs, including aliphatic and aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), PCBs and PCDD/Fs (detailed results to be reported in a later paper). Preliminary results indicate that the PCDD/F levels obtained for the soil and sediment samples are not much above 10 ng TEQ/kg. Of the more than 68 samples collected, only 14 samples indicated the presence of PDCC/Fs. The highest result obtained was 76 ng TEQ/kg for 1,2,3,7,8-PeCDD. These environmental samples are an indication of the levels of contamination to be expected in and around South Africa, emphasizing the need to make accurate measurements for these highly toxic contaminants in the South African environment at extremely low concentration levels.

4. Conclusions

GC × GC–TOFMS is a viable tool for dioxin screening and quantitation, making it suitable for environmental applications especially in cases where PCDD/F levels are greater than 1 ng kg^{-1}. The technique is ideal for application in developing countries where GC–HRMS is not available, and can be used to minimise costs by selecting only positive samples for further overseas analysis by GC–HRMS.

The Rtx-XLB/Rtx-200 column combination provides excellent separation of the compounds mandated for analysis by US EPA Method 1613. Using a multi-step temperature program (Table 1) all seventeen PCDD and PCDF components mandated by EPA Method 1613 can be separated, even the HeCDD/F isomers, thus ensuring that the final sample TEQ value can be accurately determined as it

is based on the individual contribution from all seventeen priority PCDD/F isomers. Initial isomer selectivity studies for the tetrachlorinated species indicate that the method adequately separates less toxic interferents in this region.

The *ChromaTOF* software provides many useful features for dioxin analysis and the method has been tested on several real samples. In the case where GC–HRMS results were available, a comparison of results between the two analytical approaches revealed good agreement.

GC × GC–TOFMS additionally provides full range mass spectra for all sample components, thus allowing for identification of non-target analytes which may be of importance, and also subsequent revisiting of archived data for detection of other compounds of concern, e.g., the brominated dioxins. It also provides additional certainty of identification by the second characteristic retention time for each target compound.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.03.050.

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