Environmental Engineering and Management Journal

December 2014, Vol.13, No. 12, 3063-3072
http://omicron.ch.tuiasi.ro/EEMJ/

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AN EVALUATION OF THE DETERMINABILITY OF LOW LEVEL DIOXIN CONCENTRATIONS BY HRGC/LRMS-NCI

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Abstract

High resolution gas chromatography coupled with high resolution mass spectrometry systems are often used for the analyses of dioxins at low concentrations in environmental sample matrices such as soil, vegetation, sediment, air and food. Since the construction and operation costs of these systems are so expensive, the most of the laboratories in the developing countries haven't the systems with high resolution mass spectrometry. Thus, cheaper systems with similar sensitivity such as high resolution gas chromatography coupled with low resolution mass spectrometry systems in negative chemical ionization mode may be beneficial to assess the dioxin pollution levels in environmental samples before the elaborate studies on dioxin contamination and stochastic risk assessment. In this manuscript, 17 toxic congeners of dioxins were analyzed by a system with low resolution mass spectrometry. The sensitivity, stability and resolution power of the system for the analysis of dioxins were investigated. The calibration experiments in 3 different periods were studied and the minimum detection limits were determined by using the labeled and unlabeled standard solutions. The differences according to operation conditions of the ion source were examined and expounded by using relative response factor and relative standard deviation values obtained from sequential injections. Finally, it was performed a sample analysis program for some local food samples with the aim of observing the feasibility of the system on food samples that have very low level dioxin concentrations such as egg and cow's milk. It was found that the systems with low resolution mass spectrometry in negative chemical ionization mode are capable to fulfill the requirements for the environmental analyses of dioxins at ppt levels, with the exception of 2,3,7,8-TCDD showing lower sensitivity in negative chemical ionization. Thus, using the systems with low resolution mass spectrometry in negative chemical ionization mode may be recommended for the some environmental studies for dioxins in the absence of the systems with high resolution. On the other hand, the proposed methodology cannot be used for the direct analysis of dioxins without improvement of 2,3,7,8-TCDD detection and quantification.

Key words: gas chromatography, mass spectrometry, negative chemical ionization, dioxins

Received: April, 2011; Revised final: October, 2012; Accepted: November, 2012

1. Introduction

The term ''dioxin'' refers to a class of structurally and chemically related halogenated aromatic hydrocarbons that includes polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and the ''dioxin-like'' polychlorinated biphenyls (PCBs) (Srogi, 2008). Both PCDDs and PCDFs have two benzene rings connected with oxygen atoms to each other (Fig. 1). While benzene rings connected with two oxygen bridges to each other in PCDDs, the commitments in PCDFs are founded with a carbon bond and an oxygen bridge (HMIP, 1996). PCDD/Fs have similar characteristics including high chemical, thermal and biological stability, low solubility in water, high solubility in lipid, high resistance to acidity and alkalinity, high bioaccumulation tendency and low vapor pressure. Because of the large number of congeners, relevant individual congeners are assigned with a toxic equivalency factor (TEF). The International Agency for Research on Cancer (IARC)

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named 2,3,7,8-tetrachlorodibenzo-p-dioxins (2,3,7,8-TCDD) as a human carcinogen. Each concentration of an individual congener in a mixture is multiplied with its TEF, and the resulting TCDD equivalents are added up and expressed as WHO endorsed toxic equivalents (WHO-TEQ) (Srogi, 2008). PCDD/Fs are found at levels as low as parts per trillion (ppt) or even parts per quadrillion (ppq) of matrix depending on the investigated biological/environmental samples (Srogi, 2007) and this low levels PCDD/Fs concentrations must be properly detected for advanced environmental monitoring and risk assessment studies on PCDD/Fs contamination.

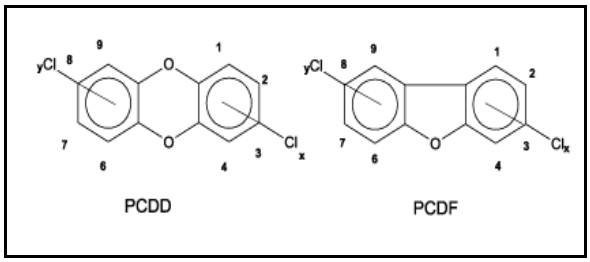

Fig. 1. General molecular structure of PCDDs and PCDFs

Four requirements were reported for the analytical procedures of PCDD/Fs measurements: (1) high sensitivity: detectable quantities and concentrations have to be in the picogram (pg) range and in the ppt range, respectively, due to the extreme toxicity of some of the compounds to be measured; (2) high selectivity: a distinction is required between PCDDs and PCDFs; a multitude of other co-extracted and possibly interfering compounds may be present at the concentrations up to several magnitudes above those of the compounds of interest; (3) high specificity: a differentiation among various isomers is desired (Buser et al., 1985) and (4) high accuracy and precision is required for congener-specific analyses of PCDD/Fs. It is also reported that a mass spectrometry (MS), especially if the MS is operating in the selected ion monitoring (SIM) mode, is ideally suited in order to meet these requirements (Srogi, 2007).

A high resolution gas chromatography coupled with a high resolution mass spectrometry (HRGC/HRMS) is used for the reference method of the determination of PCDD/Fs at low concentrations. Since this instrument is quite expensive to purchase and maintain (Rong et al., 2010), alternative instruments are usually used with more economic ion traps or quadrupole mass spectrometers (Helen et al., 2001). The capillary GC is the technique used by the most of the laboratories to determine PCDD/Fs. Columns with different stationary phases (e.g., DB-5, SP-2331, DB-Dioxin) are commonly used but several problems related to the co-elution of some of the congener's isomers have been reported (Bacher and Ballschmiter, 1992; Eljarrat and Barcelo, 2002).

Although the electron ionization (EI) is the most widely used ionization method, the negative chemical ionization (NCI) with methane as reagent gas may also be used to increase the sensitivity. In recent years, tandem ion-trap MS has been proposed as an alternative technique to HRMS for the analysis of PCDD/Fs (Splendore et al., 1997). Quadrupole ion-trap mass spectrometry (QITMS) has been largely reported as a lower-cost alternative to HRMS technique. Results from different complex matrices, such as milk, fish and fly ash showed a remarkable reliability when comparing with the results obtained from HRMS. Furthermore, in some cases MS/MS has been pointed as more selective than HRMS, whilst in some others HRMS led to better results (Fabrellas et al., 2004). However, further studies related to the precision, sensitivity and selectivity of this technique are required. The quantification is generally carried out by the isotopic dilution method, using isotope-labeled $^{13}C_{12}$ analogues. Using an isotope-labeled standard has the additional advantages including having similar elution patterns, and offering improved selectivity and sensitivity. Limits of detection down to 10-200 fg have been obtained using the most recent high-resolution MS systems (Santos and Galceran, 2002).

The electron capture detector (ECD) response of a chlorinated compound depends on both the number of chlorine atoms and their positions in the molecule. When using a quadrupole instrument with EI and selected ion monitoring (SIM), detection limits are 1–10 pg for the tetra- to hepta-chlorinated compounds and 10–50 pg for OCDD/F and DCB. Current high resolution instruments with EI and SIM operating at a resolution of 5,000–10,000 are capable of decreasing the detection limit down to 10–200 fg for the tetra- through octa-CDDs and CDFs. Using low-resolution instruments in the NCI mode with methane as reagent gas, detection limits are usually in the order of 10–100 fg range using SIM for all PCDFs (tetra- to octa-CDF) as well as for the higher chlorinated PCDDs (penta- to octa-CDD) and PCBs. This is 10–100-fold better than under EI conditions and more than 10-fold better than a GC/ECD. However, the NCI mode has a relatively poor sensitivity for 2,3,7,8-TCDD (Liem, 1999). The benefit of the NCI compared to the positive chemical ionization (PCI) and EI ionization modes is that the detection limits are significantly lower. On the other hand, there are some problems required to be solved in the NCI, e.g., low response to 2,3,7,8-TCDD. The information on this issue is contradictory and could be explained by the poor reproducibility of the NCI mass spectra (NCIMS). Mass spectra composition and detection limits are dependent on many factors such as the type of ion source, the source temperature and pressure, the reagent gas used and its purity. However, further investigations are important because the NCIMS is a promising means for the ultrasensitive determination of the chlorinated compounds in complex matrices. In the overwhelming majority of the studies, methane is used as reagent gas that provides the maximal sensitivity of PCDD/F and PCB determination, but often structural information is lacking (Chernetsova

et al., 2002). The details of NCI can be examined in Chernetsova et al. (2002).

Several significant studies have been published on the NCI applications for PCDD/Fs since 1976 (Hass, 1978) which was the first year suggesting that the NCI could increase the sensitivity and selectivity for 2,3,7,8 TCDD. Despite the fact that there are several studies on the concentrations (Fytianos and Schröder, 1997), behaviors and relative response factor (RRF) values of PCDD/Fs in various instrumental systems (Bell and Gara, 1985; Patterson et al., 1986; Schimmel et al., 1993; Viau and Karasek, 1983; Wiedmann et al., 1998), only limited number of those is interested in the NCI (Lundgren et al., 2004; Rappe et al., 1983; Waddell et al., 1987). There is a lack of investigation for the NCI compared to other system applications for the analysis of PCDD/Fs. Thus, a detailed research on the NCI is required.

The purpose of this study was to determine and evaluate the system responses of NCI to PCDD/Fs congeners in order to compare with the literature data on the analysis of PCDD/Fs by NCI. In the study, the response of a HRGC/LRMS system equipped with NCI to 17 congeners of PCDD/Fs was examined. A standard solution containing 17 unlabeled and 5 labeled dioxin congeners was sequentially injected into the HRGC/LRMS-NCI system three times in different concentrations and calibration periods.

At the end of the periods, retention times and response factors derived from the injections were evaluated in terms of system performance. Also, the factors leading to the discrepancies observed in different periods were discussed. Finally, it was performed a sample analysis program for some local food samples with the aim of observing the feasibility of the HRGC/LRMS-NCI on food samples that is known that has very low level PCDD/Fs concentrations.

2. Materials and methods

The system used in the study consists of a HP 6890 HRGC coupled with a HP 5973N LRMS equipped with a NCI module. Operation conditions of the system are given below:

- Column Flow Type : Constant Flow
- MS Ionization Method : Negative Chemical Ionization (NCI)
- Ion Source Temperature : 150 ^{o}C (2.1×10^{-4} torr source pressure for each period)
- Carrier Gas : Helium
- Carrier Gas Flow Ratio : 1.7 mL/min
- Carrier Gas Flow Pressure: 35.9 psi
- Reagent Gas : Methane
- Injection Type : Splitless
- Injection Temperature : 270^{o}C
- Injection Volume (Fixed): 2µL
- Interface Temperature : 280 ^{o}C
- Quadrupole Temperature : 106 ^{o}C
- Temperature Program : 180 ^{o}C (2 min), 5 ^{o}C/min 180 ^{o}C to 220 ^{o}C (2 min), 2,5 ^{o}C/min 220 ^{o}C to 270 ^{o}C (40 min)
- Separation Column : DB-Dioxin (60 m length, 0.25 mm ID and 0.25 µm film thickness)
- Monitoring Mode : SIM ($[M]^{\bullet-}$, $[M+2]^{\bullet-}$, $[M-2Cl]^{\bullet-}$, $[M-Cl]^{-}$ depending on the congener)

The first objective of the study was to determine the best applicable analytical method that can clearly separate and identify the 17 PCDD/F congeners in HRGC/LRMS-NCI. A standard solution mixture (purchased from Cambridge Isotopes Laboratories - Andover, MA) including 17 unlabeled (2,3,7,8 TCDD, 1,2,3,4,7,8 HxCDD, 1,2,3,6,7,8 HxCDD, 1,2,3,7,8,9 HxCDD, 1,2,3,4,6,7,8 HpCDD, OCDD, OCDF, 2,3,7,8 TCDF, 1,2,3,7,8 PeCDF, 1,2,3,7,8 PeCDD, 2,3,4,7,8 PeCDF, 1,2,3,4,7,8 HxCDF, 1,2,3,6,7,8 HxCDF, 2,3,4,6,7,8 HxCDF, 1,2,3,7,8,9 HxCDF, 1,2,3,4,6,7,8 HpCDF, 1,2,3,4,7,8,9 HpCDF-EPA 8290 EDF 5008 native standard solution) and 5 labeled (2,3,7,8 TCDD-$^{13}C_{12}$, 1,2,3,6,7,8 HxCDD-$^{13}C_{12}$, OCDD-$^{13}C_{12}$, 2,3,7,8 TCDF-$^{13}C_{12}$, 1,2,3,4,6,7,8 HpCDF-$^{13}C_{12}$ – EPA 8280 EDF 2520 internal standard solution) PCDD/Fs compounds was injected to the system, and the responses of the system were observed (Fig. 2). After obtaining the suitable chromatograms and peak shapes, the %10 valley method (U. S. EPA, 1994) was applied to all of the neighbor peaks insuring that the peak resolutions was sufficient for the analysis of PCDD/F congeners.

In order to define the sensitivity variation and linearity of the system for PCDD/F congeners in different cases (for 3 different periods), the calibration studies were performed and submitted. After the first period (Period 1), the system was shut down and the ion source was cleaned up according to a cleaning procedure (clean with alumina powder and wash in an ultrasonic bath with deionized water, methanol, acetone and hexane over 10 minute, respectively). Then, the system was maintained and the ion source was replaced. On the other hand, no changes were made between second and third periods. In every period, the standard solution was diluted with n-nonan (MERCK, reference substance for GC) to five dilution ratios (1/200, 1/100, 1/80, 1/40 and 1/20). After the dilution, the solutions were sequentially injected three times to the system in every period beginning with the 1/200 ratio. Concerning to determine the linearity and reliability of the peaks obtained during the injections, 5-point calibration curves derived (March et al., 2000) by the geometrical averages of abundances of the three injections were established for each congener.

On the other hand, in order to examine the column performance for the congeners, the values of relative retention time (RRT) were used. The values of RRF and relative standard deviation (RSD) were calculated for every peak to verify the stabilities of the congener peaks. The RRT, RRF and RSD values were calculated by the method given by U.S. EPA (2007).

The relationships of internal standard components to unlabeled components used to calculate RRT, RRF and RSD values are shown in Table 1. At the end of the periods, a detection limit (LOD) and quantification limit (LOQ) survey was conducted to determine the system sensitivities for PCDD/Fs by calculating at a minimum signal-to-noise (S/N) ratio of 3 for LOD and 10 for LOQ. The S/N ratio was calculated by the manual determination method given by U.S. EPA (1994). The methodology used in the study is illustrated in Fig. 3.

In final section of the study, it was performed a sample analysis program for some local food samples with the aim of observing the feasibility of the HRGC/LRMS-NCI on food samples that have very low level PCDD/Fs concentrations. For this purpose, two local eggs and two local cow's milk samples were analyzed.

The samples were collected in Kocaeli, one of the highly polluted cities in Turkey. From the some previous studies, it is known that the sampling area 1 (Region 1) is one of the most contaminated districts in the city. On the contrary, the sampling area 2 (Region 2) is relatively less contaminated from the other. The used analytical procedure for real world sample preparations and analyses was represented in detail by Aslan et al. (2010).

3. Results and discussion

The calibration equations and R^2 values for the dioxin congeners were presented in Table 2. For 2,3,7,8-TCDD, no significant peak (i.e., S/N>3) was observed in all the injections.

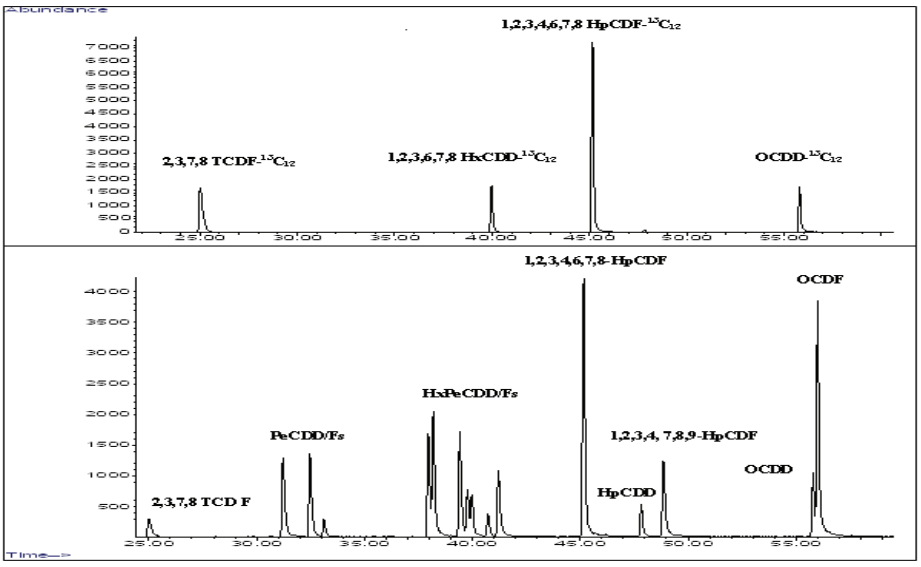

Fig. 2. A chromatogram sample of the standard solution used in the study

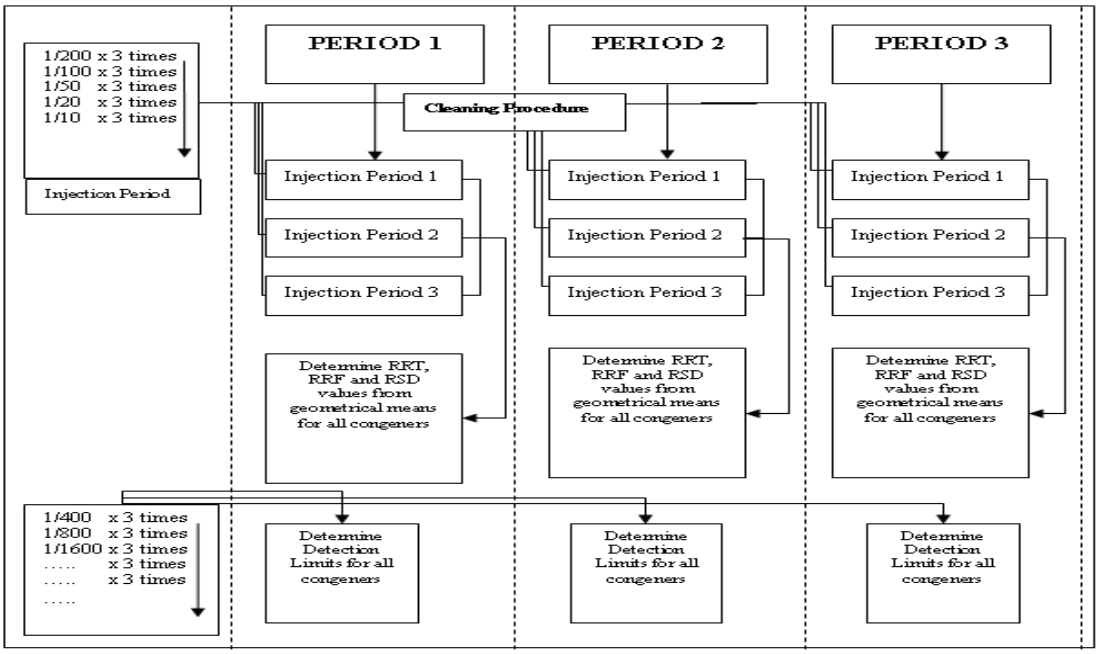

Fig. 3. Methodology used in the study

Table 1. The relationships of internal standard components to unlabeled components used to calculate RRT, RRF and RSD values

INTERNAL STANDART vs. UNLABELED CONGENERS		
INTERNAL STANDART	**UNLABELED CONGENER**	**WHO TEF**
2,3,7,8 TCDD-$^{13}C_{12}$	2,3,7,8 TCDD	1
1,2,3,6,7,8 HxCDD-$^{13}C_{12}$	1,2,3,4,7,8 HxCDD	0.1
	1,2,3,6,7,8 HxCDD	0.1
	1,2,3,7,8,9 HxCDD	0.1
	1,2,3,4,6,7,8 HpCDD	0.01
OCDD-$^{13}C_{12}$	OCDD	0.0003
	OCDF	0.0003
2,3,7,8 TCDF-$^{13}C_{12}$	2,3,7,8 TCDF	0.1
	1,2,3,7,8 PeCDF	0.03
	1,2,3,7,8 PeCDD	1
	2,3,4,7,8 PeCDF	0.3
1,2,3,4,6,7,8 HpCDF-$^{13}C_{12}$	1,2,3,4,7,8 HxCDF	0.1
	1,2,3,6,7,8 HxCDF	0.1
	2,3,4,6,7,8 HxCDF	0.1
	1,2,3,7,8,9 HxCDF	0.1
	1,2,3,4,6,7,8 HpCDF	0.01
	1,2,3,4,7,8,9 HpCDF	0.01

Table 2. Equations and R^2 values of calibration curves

*CONGENERS **	*CALIBRATION PERIODS*					
Sequence	**Period I**		**Period II**		**Period III**	
	Equation	**R^2**	**Equation**	**R^2**	**Equation**	**R^2**
2,3,7,8 TCDF	y = 8273x	0.9960	y = 26840x	0.9261	y = 6380.9x	0.9851
1,2,3,7,8 PeCDF	y = 25188x	0.9974	y=119963x	0.9578	y = 24538x	0.9938
1,2,3,7,8 PeCDD	y = 7716.1x	0.9923	y = 33099x	0.9460	y = 6580.6x	0.9839
2,3,4,7,8 PeCDF	y = 26440x	0.9854	y=118041x	0.9275	y = 26819x	0.9871
1,2,3,4,7,8 HxCDF	y = 70273x	0.9987	y=139841x	0.9388	y = 28991x	0.9886
1,2,3,6,7,8 HxCDF	y = 70273x	0.9987	y=153345x	0.9443	y = 31221x	0.9886
1,2,3,4,7,8 HxCDD	y = 23436x	0.9885	y = 54858x	0.9499	y = 9723.9x	0.9887
1,2,3,6,7,8 HxCDD	y = 23436x	0.9885	y = 42544x	0.9537	y = 7830.2x	0.9877
1,2,3,7,8,9 HxCDD	y = 6897.2x	0.9926	y = 25509x	0.9586	y = 4801.2x	0.9910
2,3,4,6,7,8 HxCDF	y = 34386x	0.9949	y=135804x	0.9452	y = 28409x	0.9896
1,2,3,7,8,9 HxCDF	y = 25585x	0.9960	y = 95482x	0.9553	y = 20030x	0.9911
1,2,3,4,6,7,8 HpCDF	y = 37872x	0.9960	y=154925x	0.9631	y = 28505x	0.9904
1,2,3,4,6,7,8 HpCDD	y = 8206.6x	0.9838	y = 27269x	0.9768	y = 4764.7x	0.9873
1,2,3,4,7,8,9 HpCDF	y = 27554x	0.9939	y = 95988x	0.9762	y = 18384x	0.9903
OCDD	y = 3783.8x	0.8069	y = 20106x	0.9809	y = 2376.3x	0.9821
OCDF	y = 28234x	0.9986	y = 76076x	0.9545	y = 13644x	0.9859

** please see Mai (2006) for the nature of the considered ions for SIM*

At the end of the calibration periods, a detection limit value (the minimum concentration corresponding to the highest dilution ratio, producing a peak with a S/N ratio higher than 3) was established for all the congeners (Table 3). The changes of detection limits (negative or positive) within the periods are presented as percentages in Table 3.

The $RRF_{max/min}$ ratios may be useful to evaluate the variations of the system responses to PCDD/Fs. Additionally, the RSD values may also be used for the same purpose. The ratios of RRF_{max}, RRF_{min} and $RRF_{max/min}$ derived from the peak areas of the congeners for 5 different dilution ratios (in turn, 1/200, 1/100, 1/80, 1/40 and 1/20) in each period were presented in Table 4.

According to the previous studies, the most significant factors reducing the system stability for PCDD/Fs in NCI are the presence of air and water in ion source, the impurities in carrier and reagent gases, and the alterations in carrier and reagent gas pressures. For a sound PCDD/Fs analysis, those factors must be controlled by checking autotune reports during the different phases of analysis. When the 28/27 ratio, the indicator of air or water leakages, is equal or higher than 5, the system stability is considered as low and the system must be shut down and cleaned. Similarly, the EMVolts value should be lower than 3000. In this study, the 28/27 ratios and EMVolts values were found to be in the ranges of 4.2-6.2 and 1576-1906, respectively.

At the end of the cleaning procedure between the periods of I and II, an increase in system sensitivity was observed as seen in Table 3 (variance after period I). On the other hand, the system stability of period II was lower than that of other periods due to the low level of R2 (see Table 3) and the high level of % RSD values (Table 4).

Table 3. Detection limits of PCDD/F congeners in NCI

CONGENERS	*Detection Limits for Period I (pg)*	*Detection Limits for Period II (pg)*	*Detection Limits for Period III (pg)*	*Variance after Period I (%)*	*Variance after Period II (%)*
2,3,7,8 TCDF	0.2500	0.0200	0.0320	+ 92	- 60
1,2,3,7,8 PeCDF	0.0860	0.0125	0.0130	+ 85	- 4
1,2,3,7,8 PeCDD	0.2600	0.0500	0.0430	+ 81	+ 14
2,3,4,7,8 PeCDF	0.0920	0.0125	0.0140	+ 86	- 12
1,2,3,4,7,8 HxCDF	0.0820	0.0125	0.0260	+ 85	- 108
1,2,3,6,7,8 HxCDF	0.0880	0.0125	0.0260	+ 86	- 108
1,2,3,4,7,8 HxCDD	0.2680	0.0125	0.0800	+ 95	- 540
1,2,3,6,7,8 HxCDD	0.3380	0.0500	0.0800	+ 85	- 60
1,2,3,7,8,9 HxCDD	0.5000	0.0500	0.0800	+ 90	- 60
2,3,4,6,7,8 HxCDF	0.1040	0.0125	0.0200	+ 88	- 60
1,2,3,7,8,9 HxCDF	0.1560	0.0500	0.0220	+ 68	+ 56
1,2,3,4,6,7,8 HpCDF	0.1080	0.0125	0.0200	+ 88	- 60
1,2,3,4,6,7,8 HpCDD	0.9600	0.0500	0.0800	+ 95	- 60
1,2,3,4,7,8,9 HpCDF	0.1920	0.0500	0.0400	+ 74	+ 20
OCDD	20	0.1000	0.2000	+ 99	- 100
OCDF	20	0.1000	0.4000	+ 99	- 300

Table 4. RRF and RSD values of PCDD/F congeners[a]

CONGENERS		*2,3,7,8 TCDF*	*1,2,3,7,8 PeCDF*	*1,2,3,7,8 PeCDD*	*2,3,4,7,8 PeCDF*	*1,2,3,4,7,8 HxCDF*	*1,2,3,6,7,8 HxCDF*	*1,2,3,4,7,8 HxCDD*	*1,2,3,6,7,8 HxCDD*	*1,2,3,7,8,9 HxCDD*	*2,3,4,6,7,8 HxCDF*	*1,2,3,7,8,9 HxCDF*	*1,2,3,4,6,7,8 HpCDF*	*1,2,3,4,6,7,8 HpCDD*	*1,2,3,4,7,8,9 HpCDF*	*OCDD*	*OCDF*
VALUES																	
Calibration I	**RRF_{max}**	1.23	1.26	1.28	1.38	1.07	1.07	1.23	1.23	1.29	1.33	1.29	1.37	1.44	1.35	1.73	1.21
	RRF_{min}	0.81	0.79	0.78	0.72	0.93	0.93	0.81	0.81	0.77	0.75	0.78	0.73	0.69	0.74	0.58	0.83
	Max/min	1.51	1.60	1.65	1.91	**1.15**	**1.15**	1.51	1.51	1.67	1.78	1.66	1.87	2.08	1.82	**2.99**	1.47
	S.D.	0.12	0.14	0.15	0.17	0.39	0.39	0.12	0.12	0.14	0.16	0.14	0.18	0.22	0.17	0.45	0.11
	Mean	1.01	1.01	1.01	1.01	1.00	1.00	1.01	1.01	1.01	1.01	1.01	1.02	1.02	1.01	1.09	1.01
	RSD (%)	11.9	13.9	14.9	16.8	39.0	39.0	11.9	11.9	13.9	15.8	13.9	17.6	21.6	16.8	**41.3**	**10.9**
Calibration II	**RRF_{max}**	1.52	1.38	1.47	1.54	1.44	1.43	1.51	1.56	1.60	1.48	1.52	1.77	1.87	1.79	2.29	2.25
	RRF_{min}	0.66	0.72	0.68	0.65	0.70	0.70	0.66	0.64	0.63	0.68	0.66	0.56	0.54	0.56	0.44	0.45
	Max/min	2.31	**1.92**	2.16	2.37	2.09	2.04	2.27	2.44	2.56	2.19	2.31	3.14	3.49	3.21	**5.22**	5.05
	S.D.	0.31	0.26	0.28	0.33	0.31	0.31	0.30	0.30	0.33	0.32	0.28	0.38	0.41	0.38	0.58	0.56
	Mean	1.04	1.03	1.03	1.05	1.04	1.04	1.04	1.04	1.05	1.04	1.04	1.06	1.07	1.06	1.13	1.15
	RSD (%)	29.9	**25.5**	27.0	31.2	29.9	29.4	29.1	29.0	31.6	30.8	26.6	35.7	38.6	35.8	**51.0**	48.7
Calibration III	**RRF_{max}**	1.46	1.52	1.81	1.42	1.40	1.38	1.47	1.57	1.39	1.37	1.39	1.38	2.41	1.52	1.38	1.20
	RRF_{min}	0.69	0.66	0.55	0.70	0.72	0.73	0.68	0.64	0.72	0.73	0.72	0.72	0.41	0.66	0.72	0.84
	Max/min	2.12	2.32	3.27	2.01	1.95	1.90	2.16	2.47	1.93	1.86	1.93	1.91	**5.83**	2.30	1.90	**1.43**
	S.D.	0.20	0.26	0.34	0.19	0.18	0.17	0.22	0.26	0.19	0.17	0.19	0.19	0.58	0.25	0.20	0.15
	Mean	1.02	1.03	1.05	1.02	1.02	1.01	1.02	1.03	1.02	1.01	1.02	1.02	1.13	1.03	1.01	1.01
	RSD (%)	19.6	25.2	32.4	18.6	17.6	16.8	21.6	25.2	18.6	16.8	18.6	18.6	**51.3**	24.3	19.8	**14.9**

[a] *Bold numbers refers to minimum and maximum values*

As mentioned above, the ion source was cleaned up and replaced between the periods of I and II. The replacement of ion source may cause air and moisture leakage in ionization chamber shortly after the start up and, the system may reveal a stability reduction. The 28/27 ratios and EMVolts values were found between 6.2 (maximum level) and 1576 (minimum level), respectively, in the autotune report obtained at the beginning of period II.

The values in autotune report obtained from the beginning of period III were obtained between 4.2 (minimum level) and 1906 (maximum level). It should be noted that there was no external intervention like cleaning, replacement, etc., in between period II - III.

The method variables (interface, quadrupole and ion source temperatures) used in this study were determined according to the literature survey, and they may be modified for new researches. The abovementioned variation was decided to be the best option for the analyses of PCDD/F congeners in our system, but no significant peak was observed for 2,3,7,8 TCDD during the experiments. This is not surprising, since the HRGC/LRMS-NCI method has very low sensitivity for 2,3,7,8 TCDD (Buser et al., 1985; Reiner et al., 2006). Modern injection

techniques such as large volume injection (LVI) can provide a solution to this problem.

The resolution power of the column and the system variables used in the study were found to be suitable for toxic PCDD/Fs congener analyses except for the congeners of OCDD and OCDF. Some co-elution problems observed for OCDD and OCDF are in accordance with the previous studies (Eljarrat and Barcelo, 2002; Nagayanagi, 2001) on the GC/MS analysis of PCDD/Fs with DB-DIOXIN capillary column. For example, an interference was observed between OCDD and OCDF-13C12 (between 1,2,3,7,8-PeCDD or 1,2,3,4,7,8-HxCDD in Eljarrat and Barcelo, 2002) peaks in a study by Nagayanagi (2001), where the same column and system were used. In the study, the chromatograms with acceptable valleys (%10 level) for octa-homologues (labeled and unlabeled) were obtained in all injections. Since, the inconsistencies observed for octa-homologues showing relatively high RRF and RSD values (see Table 4) in chromatograms may create problems, they must be controlled frequently. The column used in this study (DB-Dioxin) has a maximum temperature limitation of 270 C0 reported by the producer. The temperatures above this limit may trigger some system problems. From this point of view, different columns having temperature limits higher than that of DB-Dioxin may provide better stability for OCDD and OCDF. For example, Mayani et al. (1997) showed that a 60-meter DB-5 capillary column was more suitable than DB-Dioxin for overcoming the poor sensitivity for 2,3,7,8 TCDD and stability for OCDD/OCDF. According to the some studies in the literature, 2,3,7,8 substituted TCDD/F congeners can be easily separated with one injection into DB-Dioxin (Bacher and Ballschmiter, 1992; Eljarrat and Barcelo, 2002; Kiviranta et al., 2001; Schuhmacher et al., 2004; Schmid et al., 2005; Wunderli et al., 2000). However, the NCI stability is significantly lower than the EI stability (Pereira, 2004). The HRGC/HRMS-EI applications with polar columns are more suitable with respect to sensitivity and stability. However, construction and operation costs of HRGC/HRMS-EI are reported to be high (Perkins, 2001).

The detection limits determined for 16 PCDD/F congeners during the calibration periods (Table 3) are generally in the range of the detection limits obtained by HRGC/HRMS (e.g., Overmeire et al., 2009), GC×GC/HR-TOFMS (e.g. Shunji et al., 2008) or GC/MS-MS (e.g. Malavia et al., 2008). Hence, it might be concluded that the injections are as sufficient as to detect the PCDD/F congeners at the low concentration levels observed in the environmental samples except for 2, 3, 7, 8-TCDD. As it can be seen in Table 3, the detection limits in period II were lower than those in period I at a rate between 74 – 99%. The highest progress was for octa-homologues. This may be a result of the sensitivity increment achieved by reducing the noise levels of the chromatograms.

This may be attributed to cleaning up the ion source shortly before the second injection period. In addition, the detection limits in period III were higher at a rate of 4 - 540 % as compared to those of period II. However, some reductions were also observed in 1,2,3,7,8 PeCDD, 1,2,3,7,8,9 HxCDF and 1,2,3,4,7,8,9 HpCDF congeners. Taking into consideration that there were no external interventions to the system between the periods of II and III, pollution of the ionization chamber and the ion source during the injections in period II may result in a decrease in the sensitivity. Therefore, periodical cleaning of the ion source may have a positive influence on the system sensitivity.

Increasing the S/N values of congener peaks in chromatograms can be done by decreasing the noise level and/or increasing the signal level. The system noise level may be decreased by the following steps: (1) periodical cleaning of the ion source with an applicable clean up procedure; (2) verifying that solvent, carrier, and reagent gases used are in sufficient impurities; (3) injecting a clean and powerful solvent to the system. On the other hand, enhancing the injection volume and reducing the peak width in a constant area can increase the peak signals. Since the injection volume for the analysis of PCDD/Fs was recommended as 2 μL, this procedure was not preferred in the study.

According to the results obtained from RRT studies, the standard deviations of RRT values based on raw ratios for the congeners in all injections were in the range of 0.0001–0.0070, 0.0001–0.0090, and 0.0001–0.0030 for the periods I, II, and III, respectively. These very low deviation results suggest that the separation processes in the column were in high stable conditions during the periods.

Lundgren et al. (2004) have concluded that the responses of HRGC/LRMS-EI systems to PCDF congeners are more stable than those of HRGC/LRMS-NCI. In their study, the highest RRFmax/RRFmin values in EI and NCI modes were 2.3 and 26, respectively. It was concluded that the stability of the application of NCI was less than that of EI. Additionally, the RSD values of tetra-homologue were the highest (EI: % 23, NCI: % 49) and the responses increased with the chlorinated degree. In this study, the highest RRFmax/RRFmin values were 2.99 (for OCDD in period I), 5.22 (for OCDD in period II), 5.83 (for 1,2,3,4,6,7,8 HpCDD in period III) and the lowest ones were 1.15 (for 1,2,3,4,7,8 HxCDF and 1,2,3,6,7,8 HxCDF in period I), 1.92 (for 1,2,3,7,8 PeCDF in period II), 1.43 (for OCDF in period III) (Table 4).

Two points may be highlighted according to these results: (1) the response of the system does not depend on the chlorinated degree, (2) the system could react better to PCDF congeners than to PCDD congeners. Furthermore, the RRF values in three calibration periods have demonstrated little fluctuations, which may be accepted as an indicator of the stability.

Table 5. Analysis results for local cow's milk and egg samples*

CONGENERS	*CONCENTRATION LEVELS (pg/g fat)*			
	E-1	*E-2*	*M-1*	*M-2*
2,3,7,8 TCDF	2.191	0.278	0.032	0.034
1,2,3,7,8 PeCDF	1.422	0.738	0.215	0.228
1,2,3,7,8 PeCDD	1.647	0.295	0.675	0.222
2,3,4,7,8 PeCDF	0.887	1.358	0.396	0.420
1,2,3,4,7,8 HxCDF	0.982	0.157	0.046	0.048
1,2,3,6,7,8 HxCDF	1.351	0.305	2.235	0.758
1,2,3,4,7,8 HxCDD	1.531	0.123	0.812	0.263
1,2,3,6,7,8 HxCDD	1.531	0.123	0.812	0.263
1,2,3,7,8,9 HxCDD	1.967	0.145	0.043	0.045
2,3,4,6,7,8 HxCDF	1.967	0.145	0.043	0.045
1,2,3,7,8,9 HxCDF	1.073	0.290	0.084	0.089
1,2,3,4,6,7,8 HpCDF	1.053	0.073	0.727	0.280
1,2,3,4,6,7,8 HpCDD	0.294	0.080	0.023	0.025
1,2,3,4,7,8,9 HpCDF	2.027	0.550	0.346	0.197
OCDD	6.912	0.283	0.083	0.087
OCDF	0.594	0.146	0.042	0.045
pg TEQ/g fat	**2.895**	**0.393**	**0.999**	**0.390**

E-1 : Egg Sample from Region 1
E-2 : Egg Sample from Region 2
M-1 : Cow's Milk Sample from Region 1
M-1 : Cow's Milk Sample from Region 2
* : *Congener concentrations below the limits of quantification (LOQ) were assumed to be LOQ/2*

When RRFmax/RRFmin and RSD values were considered together, the coupling for minimum and maximum values was consistent with each other except for the minimum values for period I (Table 4).

This may be due to the cleaning the ion source after period I, which affected the system stability positively. The analysis results for local cow's milk and egg samples are presented in Table 5. According to the results obtained from analysis program, the HRGC/LRMS-NCI method had the determination capability for all congeners in the food samples such as egg and milk, except 2,3,7,8-TCDD. From the Table 5, it can be seen that the Region 1 was more contaminated than Region 2 by PCDD/Fs. This kind of results may be useful to compare a great number of the data about local PCDD/Fs contamination levels.

It can be concluded that HRGC/LRMS-NCI systems which have been used for the analyses of some persistent organic pollutants such as PCBs (Grassi et al., 2010) and PBDEs (Alaee, 2003; Eljarrat et al., 2005) can also provide a sufficiently good resolution, sensitivity and stability for the separation and analysis of 2,3,7,8-substituted PCDD/Fs except for 2,3,7,8-TCDD in the environmental samples, when the system conditions are kept in required operation levels. Although HRGC/LRMS-NCI may be taken as an "old fashion" technology, the results showed that the detection limits, sensitivity and reliability for PCDD/Fs congeners may be in the similar degrees with HRGC/HRMS except for 2,3,7,8-TCDD. In this study, a low sensitivity for 2,3,7,8 TCDD congener (the only toxic congener with no chlorine atom in the peri position "1,4,6,9 substituted" (Hass et al., 1979)) was obtained in the HRGC/LRMS-NCI system, in accordance with the literature. Therefore, further studies focusing on low sensitivity for tetra-homologue in the HRGC/LRMS-NCI systems may be recommended for cheaper PCDD/F analyses. Furthermore, using HRGC/LRMS-NCI systems for 2,3,7,8-substituted congeners except for 2,3,7,8-TCDD (Aslan et al., 2010; Karademir et al., 2013; Aguilar et al., 2014) alternately with EI mode with higher sensitivity to 2,3,7,8-TCDD (Reiner, 2010) may provide a good alternative for the analysis of PCDD/Fs in trace levels. Considering of the high costs of the HRGC/HRMS, using the HRGC/LRMS-NCI may be a valuable attempt to assess the spatial patterns and contamination levels (Weseloh et al., 2006) for PCDD/Fs and other organo-halogenated compounds simultaneously in environmental samples, before the detailed monitoring and stochastic risk assessment studies. After the fixing of high polluted areas or sample matrices by the help of HRGC/LRMS-NCI analysis, HRGC/HRMS analysis may ensure the detailed studies. In this wise, high monitoring and risk assessment costs for PCDD/Fs may be minimized.

4. Conclusions

In conclusion, the HRGC/LRMS-NCI systems are capable to fulfill the requirements for the environmental analyses of PCDD/Fs at ppt levels, with the exception of 2,3,7,8-TCDD showing lower sensitivity in NCI, when the system conditions are kept in required operation levels through an effective ion source cleaning procedure. Thus, using the HRGC/LRMS-NCI systems may be recommended

for the PCDD/Fs monitoring and stochastic risk assessment studies in the absence of HRGC/HRMS systems. On the other hand, the HRGC/LRMS-NCI cannot be used for the direct analysis of dioxins without improvement of 2,3,7,8-TCDD detection and quantification.

Acknowledgements
This study is financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (Project No. 104O121).

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