OPTIMIZATION OF DIOXIN AND FURAN ANALYSIS BY HRGC-HRMS FROM DIFFERENT TYPES OF ENVIRONMENTAL MATRICES

Andrei Niculae [1], Liliana Cruceru [1]

[1] *National Research and Development Institute for Industrial Ecology-ECOIND, Drumul Podu Dambovitei Street, 71-73, Sector 6, Bucharest, Romania*

Abstract

Dioxins and furans analysis by HRGC-HRMS for determination of PCDD's and PCDF's congeners enforce difficult preparation steps because of the possible presence of PCB's congeners, PAH and other dioxin-like compounds . Standardizated methods like ISO 1948 and EPA 8290 are very similar for air emissions, but in practice is a must to optimize every sample preparation step. In this paper are presented the experimental results obtained for optimization of the sample preparation steps which include the importance of humidity for XAD-2 adsorbent, before and after sampling, timing of addition for sampling and extraction standards in the acid treatment step, the critical role of purification adsorbents activation by temperature and even the importance of concentration of the extract between sample preparation steps. The instrumental parameters for the HRGC-HRMS, sample injection volume, chromatographic separation, mass spectrometer resolution and sensitivity, were optimized to assure the quantification of the PCDD and PCDF congeners, below national and international legislative requirements. The method was verified by analyses of CRMs, fortified samples and real samples of air emission, soil and sludge.

Keywords: *Dioxin, furan, emissions, soil, sludge.*

1. Introduction

In the last two decades, the POP's control in environment became a international strategy. JRC – the European Commission's Science and Knowledge service, presents every year a list of new organic compounds with possible toxic effects concerning population and environment. However, old POP's, especially polyhalogenated organic compounds such organochlorinated pesticides, polychlorinated biphenyls, polybrominated biphenyls and polychlorinaded dioxins and furans, are still under strictly surveillance although the industrial production of these compounds was suspended years ago. Still, these organic compounds are found in environment because of their difficult biodegradability. For this reason, in the last decade, the maximum limits for POP, have been decreased drastically and the monitoring of these compounds became more difficult. One class of these compounds are polychlorinated dioxins and furans which can still be found in environment after more then twenty years of strategies to decrease their presence. The PCDD/F analyses impose specific sample preparation steps to achieve low matrix effects and high recoveries for the specific compounds. For this reason, all the sample preparation steps are important starting with the sampling and ending with the concentration steps of the extract. To achieve lower quantification limits, all standardized method imply the use of gas chromatographs coupled with high-resolution mass spectrometers, to be able to discriminate between similar compounds such PCBs or polychlorinated PAHs, which accompany the PCDD/Fs in almost every type of environmental matrix. In this paper, were proposed to verify all the sample preparation steps to be able to prove the validity

the analytical method for the quantification of PCDD/Fs. One of the main purposes of this paper was to achieve satifactory recoveries at every preparation step and for this reason, recovery checks have been done by analyzing the concentrated extract after every sample treatment.

2. Materials

For conducted tests, were used analytical standards from LGC, EN-1948 calibration solution (CS1-CS5) EDF-4947, EN-1948 sampling standard solution EF-4138 and EN-1948 syringe standard ED-4140. Also, were used high purity solvents from LGC, picograde, toluene SO-1350, acetone SO-1142, n-hexane SO-1244 and dichloromethane SO-1185. XAD-2 adsorbent purchased from Sigma-Aldrich 10357 Supelco, diatomaceous earth from Sigma-Aldrich D3877 and as keeper, have been used n-nonane from LGC SO-1271. For the cleanup steps, were purchased bulk adsorbents, silica gel 70-230 mesh from Sigma-Aldrich 60741, Florisil 100-200 mesh from Alfa Aesar B21870 and basic alumina from VWR 21012.297. For the needed concentration steps, was used a rotary evaporator, Laborota 4003 from Heidolph. To measure analytical standards volumes, microsyringes were used, from SGE Analytical Science. For extraction phase, has been used Soxhlet glassware installation and calibrated flasks from Isolab.

3. Instrumentation and analytical method

For chromatographic analysis, has been used a gas chromatograph Trace Ultra coupled with high-resolution mass spectrometer DFS, from Thermo Scientific. For automatic injections was used an autosampler, Triplus AS from Thermo Scientific. The gas chromatograph was equipped with a split/splitless inlet. The separation had been made by a capillary column ZB-5HT Inferno of 60 m with inner diameter of 0.25 mm and 0.25 µm stationary phase, from Phenomenex. The HRGC/HRMS method parameters were optimized to comply with the standardized methods [1,2] and to satisfy the imposed resolution of 10000. The temperature of inlet was set to 250 ^{0}C, in splitless mode for 1 minute. Helium 6.0 was used as carrier gas. The column oven had a temperature program starting from 150 ^{0}C (2 minutes) with first temperature ramp of 30 ^{0}C /min. to 225 ^{0}C (17 minutes), the second temperature ramp of 5 ^{0}C /min. to 240 ^{0}C (7 minutes), the third temperature ramp of 5 ^{0}C /min. to 310 ^{0}C (7 minutes). The column flow was set to constant flow at 1 mL/min. The temperature of the transfer line was set to 260 ^{0}C. The mass spectrometer had been used in high-resolution MID mode (multiple ion detection), aiming to monitor the specific ions and relative abundance for native and labeled PCDD/F, presented in Table 1. The external calibration of the high resolution mass spectrometer was made using FC43 (perfluorotertbutylamine) and also for establish the correction of mass for the scanning analysis, calibration ions (CALI) and locking ions (LOCK). The external calibration before every analysis were done to assure a resolution of 10.000 for native and labeled PCDD and PCDF. Also, the cross-contamination and carry-over were verified by blanks of used glassware and clean blanks, to which was added internal standards.

Table 1. Specific ions for native and labeled PCDD and PCDF

Ion ID	Elemental Composition	Analyte	Ion ID	Elemental Composition	Analyte
M	$C_{12}H_4{}^{35}Cl_4O$	TCDF	M	$^{13}C_{12}H_2{}^{35}Cl_6O$	HxCDF (S)
M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF (S)
LOCK	C_6NF_{12}	FC43	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD
M	$^{13}C_{12}H_4{}^{35}Cl_4O$	TCDF (S)	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD
M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF (S)	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD (S)
M	$C_{12}H4{}^{35}Cl_4O_2$	TCDD	M+4	$^{13}C12H2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD (S)
M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD	CALI	C_8NF_{16}	FC43
M	$^{13}C_12H4{}^{35}Cl4O2$	TCDD (S)	M+2	$C_{12}H{}^{35}Cl_6{}^{37}ClO$	HpCDF
M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD (S)	M+4	$C12H{}^{35}Cl5{}^{37}Cl2O$	HpCDF
M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF	LOCK	C_8NF_{16}	FC43
M+4	$C_{12}H_3{}^{35}Cl3{}^{37}Cl_2O$	PeCDF	M	$^{13}C_{12}H{}^{35}Cl_7O$	HpCDF (S)
CALI	C_7NF_{14}	FC43	M+2	$^{13}C_{12}H{}^{35}Cl_6{}^{37}ClO$	HpCDF
LOCK	C_6NF_{12}	FC43	M+2	$C_{12}H{}^{35}Cl_6{}^{37}ClO_2$	HpCDD
M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF	M+4	$C_{12}H{}^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD
M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF	M+2	$^{13}C_{12}H{}^{35}Cl_6{}^{37}ClO_2$	HpCDD (S)
M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF (S)	M+4	$^{13}C_{12}H{}^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD (S)
M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF (S)	CALI	C_9NF_{18}	FC43
M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD	LOCK	C_8NF_{16}	FC43
M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO$	OCDF
CALI	C_7NF_{14}	FC43	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O$	OCDF
M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD (S)	M	$C_{12}{}^{35}Cl_8O_2$	OCDF
M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD (S)	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD
LOCK	C_7NF_{14}	FC43	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD
M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF	CALI	C_9NF_{18}	FC43
M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O$	HxCDF	M+2	$^{13}C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD (S)

4. Experimental

First of all, the HRGC-HRMS was calibrated between 0.1 and 200 ng/L native PCDD/PCDF, using the calibration kit EN-1948 Calibration solution (CS1-CS5) from LGC Standards. Some examples of linear regressions obtained after calibration are presented in Figure 1. The calibration was checked by a solution of 100 ng/L obtained after 1:2 dilution of CS5 calibration solution. One of the most important steps in PCDD and PCDF congener analysis is the cleanup procedure, were the influence of matrix can lead to difficult or impossible quantification analysis. First of all, adsorbents have

been thermal activated at needed temperature and time, treated with acid or added water, mixed for 24 hours and stored in brown sealed glassware [1,2]. The experimental tests were divided in five categories; The extraction - where was verified the numbers of extraction cycles needed to achieve a recovery greater than 80 percent, the cleanup steps were considered as single category and verified the needed solvent volumes for complete elution of PCDD and PCDF congeners, for acid silica gel, basic alumina and hydrated florisil. The last testing category was the concentration step, implicated after each procedure to reduce the extract volume to 25 µL. To eliminate the evaporation of the all extract, a keeper had to be used with high boiling point., In this case, n-nonane was the optimal choice as a keeper, with a boiling point of 151 ^{0}C.

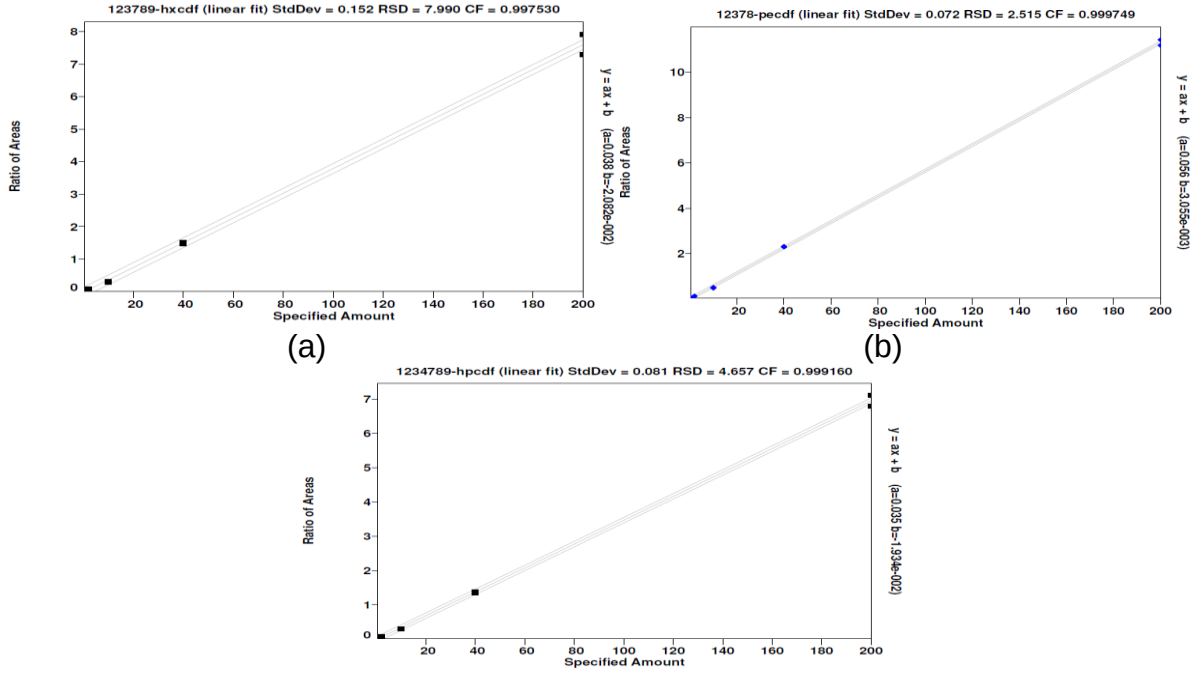


Figure 1. Linear regressions for 1,2,3,7,8-PeCDF (a), 1,2,3,7,8,9-HxCDF (b), 1,2,3,4,7,8,9-HpCDF(c)

The Soxhlet extraction was verified for emission samples, where XAD-2 was used for sampling and for solid samples was used diatomaceous soil. Before extraction, sampling standards were added. Toluene was used as extraction solvent and three extraction times 8, 16 and 24 hours, were tested. After each extraction time, 25 µL of n-nonane were added as keeper. The extract was concentrated by rotary evaporator to the keeper volume. The syringe standard was added and the final sample was transferred in a low volume glass insertion of 100 µL and analyzed by HRGC-HRMS. In Figure 2, can be observed an example of the separation of labeled internal standards part of sampling and syringe standards.

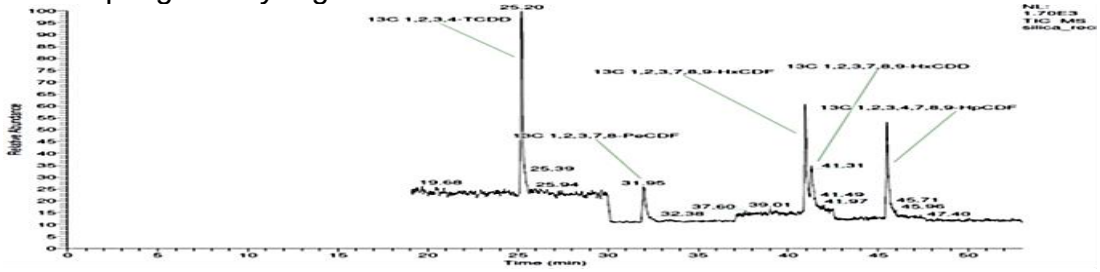


Figure 2. Chromatogram of labeled internal standard contained in sampling and syringe standards

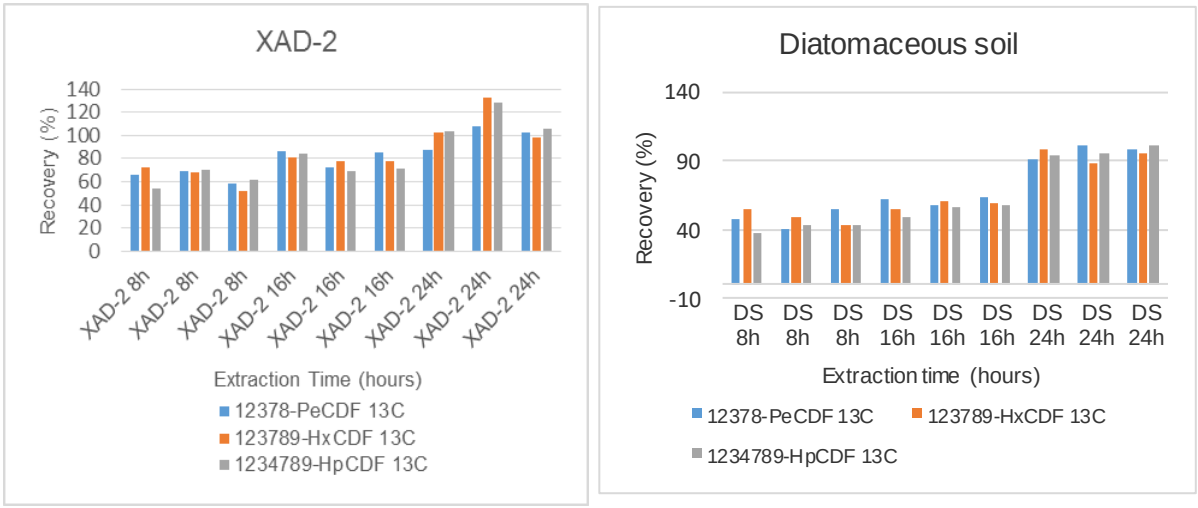
The glass columns used for cleanup steps had 17 cm length and 15 mm internal diameter. For the 44 % acid silica gel cleanup step, 6 g of adsorbant were used and 1.5 g of anhydrous sodium sulfate. The columns were pre-cleaned with 20 mL of n-hexane. Before the solvent reach the sulfate layer, the sample as 50 µL of sampling standards was added. The elution of PCDD and PCDF congeners was tested at three different n-hexane volumes, 15, 25 and 40 mL.

The elute was collected in 100 mL conical flasks, 25 µL of keeper was added and concentrated to the keeper volume. After concentration 50 µL of the syringe standard was added and the final samples were transferred in low volume glass insertions. The basic alumina cleanup has been made using 2 g of adsorbent and a superior layer of 1.5 g anhydrous sodium sulfate. The column was pre-cleaned with 20 mL of n-hexane. Before the the solvent reach the sulfate layer, the sample as 50 µL of sampling standards was added. The elution of PCDD and PCDF congeners was tested at three different dichlorometane volumes, 15, 25 and 40 mL. The elute was collected in 100 mL conical flasks, 25 µL of keeper was added and concentrated to the keeper volume. After concentration 50 µL of the syringe standard was added and the final samples was transferred in low volume glass insertions. The 5% H_2O florisil cleanup has been made using 2.5 g of adsorbent and a superior layer of 1.5 g anhydrous sodium sulfate.

The column was pre-cleaned with 20 mL of n-hexane. Before the solvent reach the sulfate layer, the sample as 50 µL of sampling standards was added. The elution of PCDD and PCDF congeners was tested at three different dichlorometane volumes, 15, 25 and 40 mL. The elute was collected in 100 mL conical flasks, 25 µL of keeper was added and concentrated to the keeper volume. After concentration 50 µL of the syringe standard was added and the final samples was transferred in low volume glass insertions. All the tests were repeated in three replicates for extractions and five replicates for cleanup steps. All the samples were analyzed in three replicates and the recovery was calculated based on the internal standards from the calibration solutions.

5. Results and Discussion

The effects of the humidity of the XAD-2 adsorbent was studied in recent paper which concluded that the non-polarity of the polymer prevent the influence of the humidity concerning the adsorbance of non-polar or semi-polar organic compounds [3]. Also, the relative humidity at sampling has a low influence to XAD-2 adsorbance as was demonstrated in previous studies of dioxin-like compounds [4]. All the optimization tests were based on the recovery of sampling standards as labeled PCDF congeners. The results for extractions of XAD-2 and diatomaceous soil are presented in Figure 3, based on the different extraction times and the relative recoveries.



(a) (b)

Figure 3. XAD-2 extraction recoveries (a) and diatomaceous soil extraction recoveries (b)

The results for the XAD-2 extraction indicate that the necessary time for recoveries over 80% is at least 16 hours [5,6] and for higher values, 24 hours [7] in compliance with literature data. The results for diatomaceous soil revealed the literature data for recoveries, for 16 hours extraction time, the recovery was below 70% [8] and for 24 hours extraction time, the recovery was greater de 85% [9]. The silica gel cleanup recoveries show a proportional trend with the eluent volume between 15 mL and 25 mL and no great difference using 40 mL. The results are presented in Figure 4.

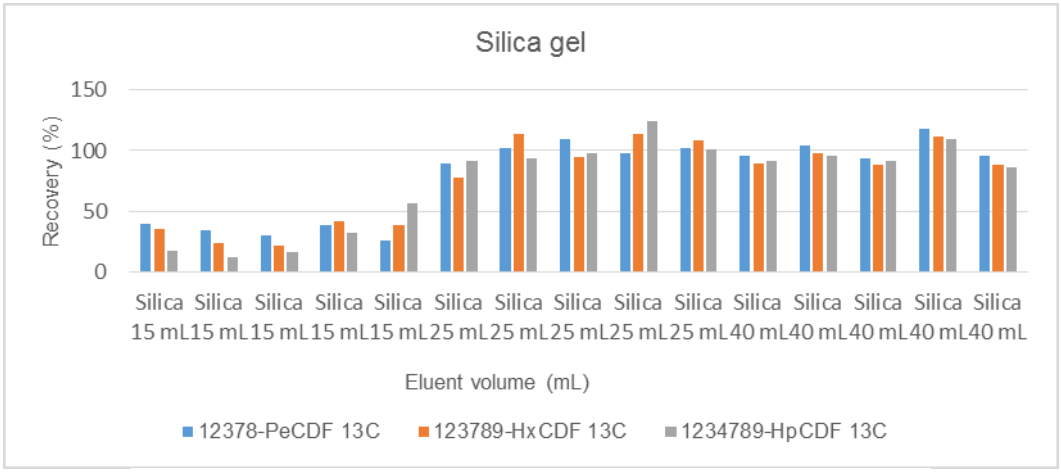


Figure 4. The silica gel recoveries at different eluent volumes.

For basic alumina tests, was observed the same tendency as for silica gel, eluent volumes between 15 mL and 25 mL shown an increase in recovery but for 40 mL dichloromethane are not relevant rises in recovery, as shown in Figure 5.

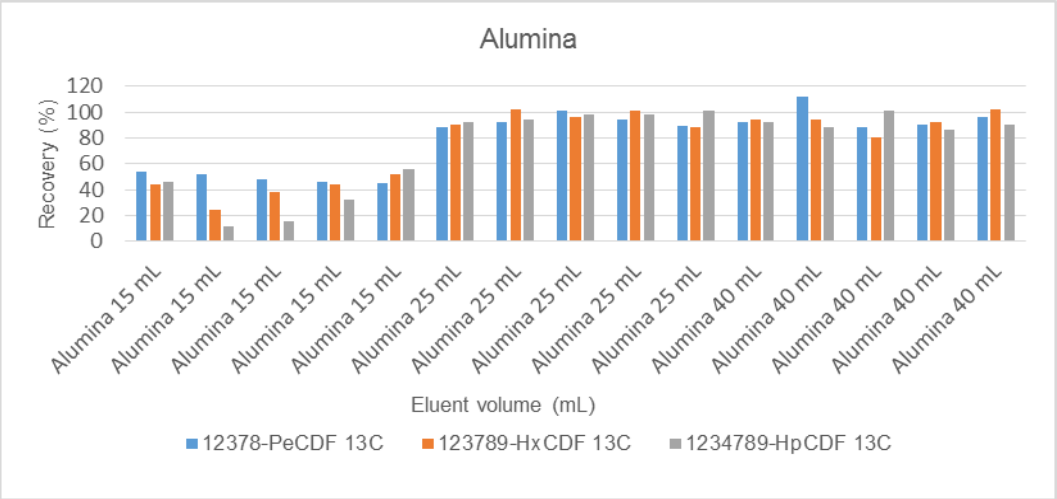


Figure 5. The alumina recoveries at different eluent volumes.

The same tendency was observed to the dichloromethane increasing volume for the hydrated florisil where the optimum elution volume was 25 mL for recoveries higher than 70%. The result are sumarized in Figure 6.

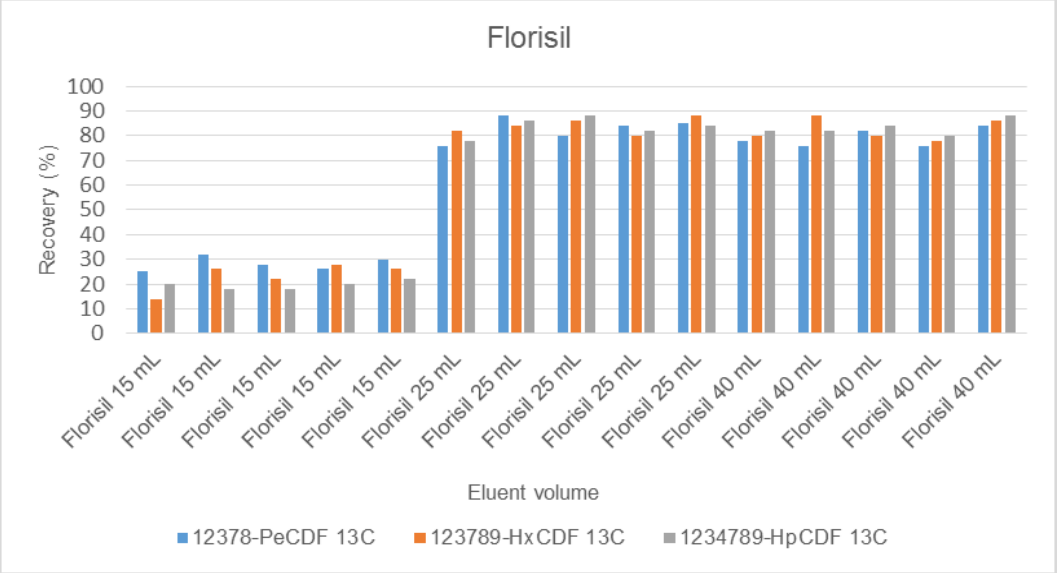


Figure 6. The florisil recoveries at different eluent volumes.

6. Conclusions

The instrumental parameters of the HRGC-HRMS were optimized for higher sensitivity and resolution, to identify and quantify the PCDD and PCDF congeners in compliance with standardized methods for different matrices analyses (air emissions, soil, sludge). The applied testing method was sufficient to evaluate the recoveries at different sample preparation steps and the repetability of results were below 5% RSD.The results of sample preparation tests revealed a concordance with literature data for extraction time but also have highlighted the importance of adsorbant quantities used for cleanup and the optimal eluent volumes.

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