Clean-up of olive oil samples with high capacity disposable columns for determination of PCDD/Fs. Comparison with sulphuric acid treatment.

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Introduction

The determination of PCDD/PCDFs at trace levels requires complicated and very time-consuming sample extraction and clean-up procedures. Methods used for the determination of PCDD/PCDFs in food samples combine sulphuric acid treatment (24-48h) with an automated clean-up system, Power Prep System^{TM} (F.M.S., Inc., USA), with three pre-packed acid/base silica gel, alumina and active carbon chromatographic columns[1,2] . This paper describes a time-saving procedure due to the avoidance of the sulphuric acid step.

Materials and Methods

Standards:

All PCDD/Fs standard solutions, EPA 1613 PAR (Precision & Recovery Stock Solution), EPA 1613 LCS (Labelled Compound Stock Solution), EPA 1613 CSS (Cleanup Standard Spiking Solution), EPA 1613 ISS (Internal Standard Spiking Solution), EPA1613 CLS (Extended Calibration, Low Level), EPA 1613 CS 0.5, EPA 1613 CS1, EPA 1613 CS2 and EPA 1613 CS3, were obtained from Wellington Laboratories (Ontario, Canada).

PAR standard solution was diluted with acetone (Riedel-de Haën, Germany, for analysis of dioxins) 1:50 (v/v) to prepare a diluted spiking solution. The amount of spiked PAR was 100µl, equal to the concentration of the native compounds of CSL. LCS was diluted with acetone (Riedel-de Haën, Germany, for analysis of dioxins) 1:50 (v/v) to prepare a diluted spiking solution. The amount of spiked LCS was 100µl. CSS was $^{37}Cl_4$-2,3,7,8-TCDD and was diluted with nonane (Fluka, Switzerland, puriss) 1:50 (v/v) to prepare a diluted spiking solution. The amount of spiked CSS was 100µl. ISS was composed of $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$ -1,2,3,7,8,9-HxCDD.

Chemicals and Reagents

All solvents employed (hexane, dichloromethane,ethyl acetate,toluene) were for pesticide residue analysis and were provided by Labscan (Dublin, Ireland).

Sulphuric acid was for analysis (95-97 %), and was obtained from Scharlau (Barcelona, Spain).

The disposable packaged columns were multi-layered (acid/base/neutral) silica columns, a basic alumina column, a carbon/celite column and a high capacity acidic silica column. All were provided by Fluid Management Systems Inc. (Watertown, USA).

Samples

Twenty samples (6g) of commercial olive oil were weighed. Each sample was spiked with known amounts of standards: 100µl of PAR, 100µl of LCS and 100µl of CSS. In this study two protocols were followed. The first one includes an acid attack. Ten samples were placed into a separatory funnel, 100ml of hexane and 50ml of sulphuric acid were added, shaking 30 seconds and afterwards left to stand for 24 hours.This step was repeated three times with 50ml of sulphuric acid and stopping for 2.5 hours each time. The obtained extracts were concentrated and diluted with hexane (18ml). Prior to loading the samples, each extract was filtered through a 1µm filter. For cleanup the samples were processed using the automated Power-prep System^{TM} (F.M.S, Inc., USA). This system was used with three disposable columns (multilayer silica, alumina and carbon/celite) in order to separate analytes of interest

from matrix interferences. The cleanup solvents were: n-hexane, 98:2 n-hexane/dichloromethane, 50:50 n-hexane/dichloromethane, 50:50 ethyl acetate/toluene and toluene. The PCDD/Fs were eluted with 75ml of toluene, then evaporated to about 1ml in a rotary evaporator, transferred to vials and then evaporated to dryness under a nitrogen stream sample concentration device. The samples were reconstituted in 10µl of ISS and 10µl of nonane.

In the second protocol, ten samples were added to 30ml of n-hexane, filtered through a 1µl filter and loaded into the Power-Prep System^{TM}. For lipid removal, an additional disposable high capacity acidic silica column was added prior to the multilayer silica column instead of the sulphuric acid treatment. The cleanup solvents were the same, but the flow and amount were changed in order to achieve the optimization of the process. The extracts were treated as described in the first protocol.

In this study, two method blanks were included in each protocol. Blank corrections were made in order to avoid the contribution of detected congeners to the recoveries of the PAR standards.

HRGC/HRMS conditions.

Analysis of tetra- to octa-CDD/Fs was performed using a Micromass (Waters) Autospec Ultima NT interfaced with an HP 6890 Series gas chromatograph (Agilent, USA). Data system was MassLynx 4.0 software. Samples were splitless-injected in a DB5-MS fused silica capillary column (J&W Scientific; 60m x 0.25mm x 0.25µm).

The Autospec was operated at minimum 10.000 resolution (10% valley definition) in EI+ mode and selected ion monitoring mode (SIM). Electron energy of approximately 33eV was used with 600µA trap current and a 280ºC source temperature. The measurement followed the general procedures of EPA method 1613B (Tetra-through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS).

GC oven temperature program:

150ºC (4min)

10ºC/min to 220ºC

4.2ºC/min to 310ºC (7min)

Carrier gas: He. Constant flow: 1.3 ml/min. Injector temp: 280ºC. Splitless injection: 1-2µl. Purge time: 2min. Purge flow: 20ml/min.

Quantification was carried out by the isotopic dilution method using relative response factors (RRF) previously obtained by analisis of standard solution mixtures (CS). The different internal standards and respective RRF were used for quantification of unlabeled compounds. The recoveries of labeled standards were calculated by use of mixtures of labeled compounds (ISS) added before HRGC/HRMS analysis. These recoveries were used to check that the analysis procedure was satisfactory. The US EPA method 1613B[1] has established acceptable recovery ranges between 40 and 120%

Results and Discussion

The average recoveries and relative standard deviations (RSD %) of the native and labelled PCDD/PCDF congeners are shown in table 1. The recoveries of the native compounds were high for both protocols, between 100% and 116% for the first protocol, and between 95% and 105% for the second protocol.

	H_2SO_4 (n=10)		HCDC (n=10)	
	AVERAGE RECOVERIES %	RSD%	AVERAGE RECOVERIES %	RSD%
2,3,7,8-TCDF	**102.16**	**3.77**	**100.88**	**7.67**
1,2,3,7,8-PeCDF	**101.74**	**5.05**	**98.71**	**5.62**

2,3,4,7,8-PeCDF	**101.36**	**5.05**	**97.52**	**6.39**
1,2,3,4,7,8-HxCDF	**102.06**	**4.99**	**99.73**	**5.53**
1,2,3,6,7,8-HxCDF	**100.68**	**5.06**	**97.14**	**4.86**
1,2,3,7,8,9-HxCDF	**100.78**	**4.07**	**98.04**	**5.07**
2,3,4,6,7,8-HxCDF	**103.12**	**4.67**	**99.07**	**5.13**
1,2,3,4,6,7,8-HpCDF	**102.63**	**4.42**	**99.90**	**5.00**
1,2,3,4,7,8,9-HpCDF	**104.36**	**6.02**	**100.98**	**4.29**
OCDF	**116.60**	**15.11**	**101.02**	**12.08**
2,3,7,8-TCDD	**100.87**	**7.35**	**94.84**	**7.55**
1,2,3,7,8-PeCDD	**107.06**	**5.25**	**100.88**	**7.05**
1,2,3,4,7,8-HxCDD	**106.87**	**5.02**	**101.36**	**7.07**
1,2,3,6,7,8-HxCDD	**106.03**	**6.08**	**99.40**	**7.14**
1,2,3,7,8,9-HxCDD	**101.21**	**4.32**	**104.83**	**7.20**
1,2,3,4,6,7,8-HpCDD	**104.87**	**4.98**	**100.06**	**5.80**
OCDD	**104.23**	**4.34**	**100.39**	**7.00**
13C-2,3,7,8-TCDF	**93.69**	**12.59**	**101.54**	**12.63**
13C-1,2,3,7,8-PeCDF	**103.19**	**11.57**	**102.01**	**13.8**
13C-2,3,4,7,8-PeCDF	**106.26**	**12.13**	**104.56**	**13.67**
13C-1,2,3,4,7,8-HxCDF	**97.64**	**13.45**	**95.12**	**13.36**
13C-1,2,3,6,7,8-HxCDF	**98.85**	**15.51**	**95.51**	**14.3**
13C-2,3,4,6,7,8-HxCDF	**97.42**	**15.87**	**93.19**	**14.99**
13C-1,2,3,7,8,9-HxCDF	**89.16**	**21.43**	**88.97**	**13.85**
13C-1,2,3,4,6,7,8-HpCDF	**83.14**	**11.04**	**77.03**	**17.87**
13C-1,2,3,4,7,8,9-HpCDF	**79.24**	**11.50**	**69.80**	**18.99**
13C-2,3,7,8-TCDD	**83.18**	**13.17**	**98.29**	**7.02**
13C-1,2,3,7,8-PeCDD	**104.90**	**9.87**	**105.47**	**5.94**
13C-1,2,3,4,7,8-HxCDD	**97.26**	**11.10**	**92.74**	**7.40**
13C-1,2,3,6,7,8-HxCDD	**95.04**	**10.09**	**91.51**	**7.49**
13C-1,2,3,4,6,7,8-HpCDD	**79.41**	**9.08**	**73.81**	**12.22**
13C-OCDD	**63.65**	**19.09**	**56.40**	**12.25**

Table 1: Recoveries for PAR and LCS standards.

In the case of the labelled compounds the recoveries were lower, between 64% and 106% for the first protocol, and 56% and 105% for the second protocol, but they were still in good agreement with the minimum requirements of well accepted methods[1].

The limits of detection (LODs) and quantification (LOQs) were similar for both protocols.

No significant differences were found for the RSDs in both cases.

The results and relative standard deviations obtained indicate that both protocols are comparable concerning their efficiency and variability for the determination of PCDD/Fs in olive oil samples.

The second protocol simplifies the sample manipulation and further reduces the sample cleanup to 3 hours. This protocol offers a nearly 48-hour reduction of sample preparation time, this being its most relevant benefit.

The use of a high capacity disposable acidic column instead the sulphuric acid attack is a viable technique for the determination of PCDD/Fs in olive oil.

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References

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