A METHOD FOR THE DETERMINATION OF PCDD/PCDF IN MILK AND DAIRY PRODUCTS

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Abstract

The target of the present work was to set up an analytical method for the determination of PCDD/PCDF in milk and dairy products. The first step consisted of a liquid-liquid extraction, by using a mixture methyl alcohol: diethyl ether: n-hexane 33:33:33 v/v, followed by a purification with a multilayer column (Na_2SO_4 anhydrous, silica gel; mix Na_2SO_4:$NaHCO_3$ 1:9 w/w; mix of 32 ml H_2SO_4 at 96% and 52 g celite (545); Na_2SO_4 anhydrous) and an alumina column. The purified extract was then analyzed by HRGC/HRMS. Since the method shows good mean recoveries for all labelled congeners introduced in the sample as syringe standards, it was possible to assume that the analyzed samples did not undergo overvalue nor underestimation of their quantitative of $^{12}C_{12}$ congeners. The specificity of the method allowed to obtain a good control of matrix effect and an accurate definition of the peaks; moreover, by modifying the dimensions of the chromatographic columns as a function of the fat quantity, purification was made really effective, and eluates were obtained which were found directly suitable for analysis in High Resolution Mass Spectrometry.

Introduction

A method for the determination of polychlorinatedibenzodioxins in milk and dairy products was set up due to an environmental pollution, in 2003, in the Campania region, close by Neaples, Italy. The contamination of some pasture lands was originated by the fall-out of products from accidental burning of waste material. At that time the Agency for the Environmental Monitoring started the examination of soils, vegetation, forages, milk, and dairy products in a coordinated fashion. The milk under analysis came directly from the farms in the contaminated localities.

Analitical Method

The analitical method was applied to 100 ml of milk with a liquid-liquid extraction and final purification by chromathographic columns followed by instrumental analysis with High Resolution Gas Chromathography/ Mass Spectrometry. All the procedures of the extraction process were developed using certified and labelled internal standards (PCDD-PCDF $^{13}C_{12}$) having the same concentration (within the legal limits).

Extraction of the fat portion from milk

The liquid-liquid extraction was obtained in a split-funnel. The amount of milk to be analyzed was in the range 70-100 ml, each quantity selected being related to the amount of fat present in the sample. The samples were of 70 ml for the buffalo milk and 100 ml for the bovine milk.

An extraction internal standards, $^{13}C_{12}$ labelled, and 1 g sodium oxalate were added to the milk and then homogenized by vigorous stirring. The solvents, listed below, were added to the milk, under stirring and degassing of the mixture:

- 100 ml methyl alcohol
- 100 ml diethyl ether
- 100 ml n-hexane

The mixture was left to stand for one hour resulting in the formation of two phases. The upper hexane phase was poured in a flask after contact with anhydrous sodium sulphate and filtration. The fat contained in this hexane phase was determined by evaporation of the solvent in rotavapor or centrifugal-evaporator and then the fat was brought to constant weight under a nitrogen flow.

Table 1 shows the average percentage of fat present in every kind of milk under exam.

Table 1- Average fat content in bovine and buffalo milk

Bovine milk	**4,6 %**
Buffalo milk	**8,5 %**

For dairy products the fat extraction techniques consisted in lyophilization and then final extraction through ASE, sonication or soxhlet.

Purification of the extract

The extracts purification was performed in the following steps:

Step 1) Purification by multilayer column

A glass column (internal diameter 4 cm) was stuffed from the bottom to the top with degreased fibreglass; 0.5 cm Na_2SO_4 anhydrous; 0.5 cm silica gel; 15 cm with a mix of Na_2SO_4:$NaHCO_3$ 1:9 w/w; 8 cm with a mix of 32 ml H_2SO_4 at 96% and 52 g celite (545); 15 cm Na_2SO_4 anhydrous.

A solution of the fat in hexane was put on the top of the column and hexane was used (280 ml) for elution. The eluted was concentrated in centrifugal-evaporator. After complete evaporation of the solvent by flowing nitrogen, the solid wasdissolved in hexane (1 ml).

Step 2 Purification by active basic alumina column

A glass column (internal diameter 1cm) was stuffed with dried basic alumina (height 10 cm), previously activated in oven at a temperature of 110-150°C and then left to cool in a drier. After washing of the column with hexane (40 ml), the hexane solution of the fat eluted from the multilayer column was totally transferred to the top of the alumina column. From the following elution, the below fractions were separated:

a) 20 ml hexane at 2% of CH_2Cl_2 (preeluate),

b) 80 ml hexane at 50% of CH_2Cl_2 (eluate) containing the products PCDD/PCDF.

The eluate was put into a flask with merlin neck, concentrated with rotavapor, and transferred (hexane) in a 4 ml vial and washings (hexane) added to it. After evaporation of hexane (bubbling of N_2), the eluate was reconstituted with syringe standards and analyzed.

Instrumental analysis

The instrumental analysis was made by using a magnetic HRGC/HRMS.

The percentage recovery of extraction labelled congeners in relationship with syringe labelled standard is of the most importance.

The Italian accredited internal analytical method – n°236 SINAL (Sistema Nazionale per l'Accreditamento dei Laboratori) and n°8 ORL (Organismo di Riconoscimento dei Laboratori) - warrants the result for a range of labelled $^{13}C_{12}$ congeners recovery between 60 and 120%. The choice of the Wellington Laboratories Inc labelled standards was due to the reason that this is the only company selling these standards to be ISO 9001:2000 certified.

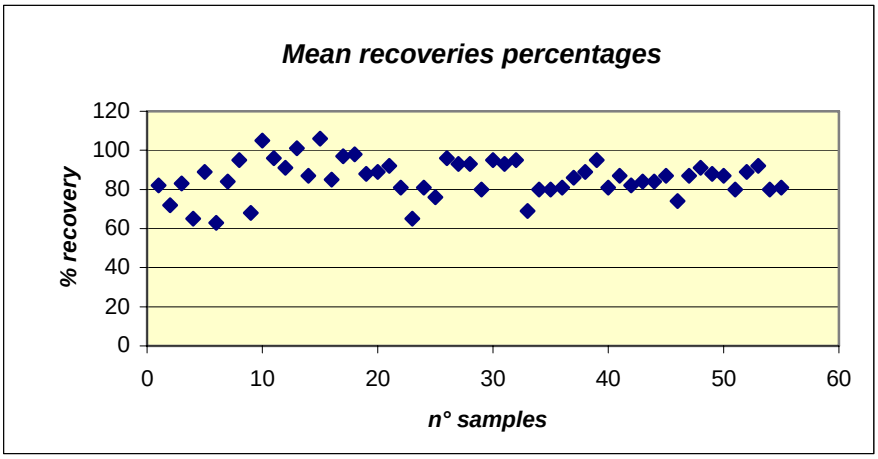
Tab 2 - Analytical results on 55 milk samples, in pg WHO-TEQ/g

Sample N°	PCDD/DF pg WHO- TEQ/g	Sample N°	PCDD/DF pg WHO- TEQ/g	Sample N°	PCDD/DF pg WHO- TEQ/g
1	**2,1**	11	**1,89**	21	**0,93**
2	**2,81**	12	**0,82**	22	**2,92**
3	**1,33**	13	**0,96**	23	**7,28**
4	**2,95**	14	**2,23**	24	**3,23**
5	**1,24**	15	**2,39**	25	**2,92**
6	**2,17**	16	**2,88**	26	**2,92**
7	**1,08**	17	**2,61**	27	**5,04**
8	**2,63**	18	**2,31**	28	**1,48**
9	**1,34**	19	**1,98**	29	**1,48**
10	**1,3**	20	**0,56**	30	**4,34**

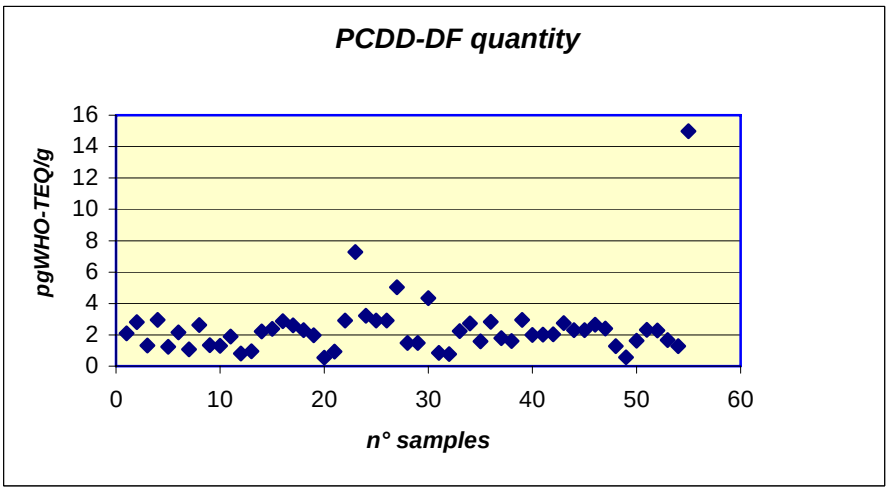
Sample N°	PCDD/DF pg WHO- TEQ/g	Sample N°	PCDD/DF pg WHO-TEQ/g	Sample N°	PCDD/DF pg WHO- TEQ/g
31	**0,86**	41	**2,02**	51	**2,32**
32	**0,77**	42	**2,03**	52	**2,29**
33	**2,24**	43	**2,76**	53	**1,68**
34	**2,73**	44	**2,31**	54	**1,29**
35	**1,59**	45	**2,31**	55	**14.9**
36	**2,84**	46	**2,65**		
37	**1,79**	47	**2,40**		
38	**1,61**	48	**1,29**		
39	**2,95**	49	**0,57**		
40	**2,00**	50	**1,64**		

Figure 1 shows the values of the average recovery percentages, all higher than 60 %.

Figure 1 – Recoveries results



Figuere 2 – Samples results



In Figure 2 the results obtained for all the samples are reported. Only four of the total number of samples examined (55) show a content of PCDD-DF exceeding the present Italian legal limit.

Conclusions

With the proposed method we obtained good mean recoveries percentages of the labelled congeners, in all the analyzed samples, as shown in Table 1. We can assume that the analyzed samples did not undergo overvalue nor underestimation of their quantitative value of $^{12}C_{12}$.

In only 4 of the analyzed samples, the content of PCDD/F was found to exceed the present Italian legal limit.

The specificity of the method allowed to obtain:

- Control of matrix effect;
- Accurate definition of the peaks.

The flexibility of the analytical method, related to the dimensions of the chromatographic columns and to the amount of fat present in the samples examined, allowed the define a really effective purification process and to obtain eluates suitable for direct analysis in High Resolution Magnetic Spectrometry, without any work increase for the instrumentation.

References

Berlincioni M, Croce G, di Domenico A, Lolini M, Pupp M, and Rizzi L (1992) *Organohalogen Compounds* 9, 33-36.

Berlincioni M, Croce G, di Domenico A, La Rocca C, Lolini M, Megli A, Pupp M, and Rizzi L (1993) *Organohalogen Compounds* 11, 41-44.

Croce G, Lolini M, Donati P, Baldassini M, Marsico AM, Martellini F, Baldassini M, Giulia Guerranti G (2001) *Bollettino dei Chimici Igienisti Anno 52° - n° 9 novembre 2001 - S4.*