

THE USE OF ACCELERATED EXTRACTION TECHNIQUES FOR CITRUS PULP PELLETS PCDD/F MONITORING PROGRAM

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Introduction

Towards the end of March 1998, the Ministry of Agriculture for Brazil was made aware of the detection of contaminated Citrus Pulp Pellets (CPP) that are used as part of the feed for dairy cows in Germany. A concerted effort was established to discover the source of the contamination, which pointed to a lime converter who provided lime milk as a raw material for the CPP production ¹.

After the identification of the source, a specific legislation was published in Brazil to control the levels of dioxins and furans in CPP and lime. Thus, an upperbound value of 500 pg/kg was established as a tolerance level for any lime that should be used in animal feed. The same level was applied for citrus pulp pellets. Over 1000 samples were analysed during the first two years of the monitoring program of Brazilian Ministry of Agriculture ². No sample showed upperbound levels higher than 500 pg/kg I-TEQ.

The program established was able to achieve the production and commercialization quality control and guarantee that both internal and external consumers are free from contaminated products. Nevertheless, several samples are taken during loading of warehouses and shipment of samples. Thus, analysis turnaround time becomes a very important task, once the costs for storage can become too elevated or even the shipment can arrive at its final destination with no results. Until now, several efforts were made to assure that this does not happen.

This study shows the tests performed during the second year of monitoring program of Brazilian Ministry of Agriculture for PCDD/F contamination. Different extraction techniques were applied to USEPA 8290 methods, reducing significantly the analysis time to less than 05 days. The most commonly used extraction method is Soxhlet extraction. It is time-consuming (18 to 36 hours) and requires large volume values of solvent, 250 to 500 mL.

Accelerated Solvent Extraction (ASE) is equivalent to U.S. EPA methods for the extraction of organochlorine pesticides, organophosphorus pesticides, semivolatiles or base, neutral, acids, chlorinated herbicides, polycyclic aromatic hydrocarbons (PAH), and polychlorinated biphenyls (PCB). ASE is a technique that uses liquid solvents at elevated temperatures and pressures. These conditions allow extractions to take place in short periods of time with small solvent volumes as compared to conventional techniques such as sonication and Soxhlet extraction.

Methods and Materials

The tests for different extraction methods were performed with 30 g CPP spiked sample (CPP sample previously analysed and not contaminated with dioxins and furans) at 2.5 ng dioxins and furans (83 pg/Kg). Sample processing was based on USEPA 8290 protocols.

Extraction conditions

In summary an accurately weighed 30g aliquot of the sample was spiked as presented. Table 1 lists the conditions used for the extraction of CPP samples. All other conditions were held constant.

Table 1: Comparative presentation of the extraction conditions used in CPP samples.

Extraction Conditions for CPP samples		
Condition	Soxhlet	ASE
Sample amount	30 g	30 g
Solvent	Dichloromethane	Dichloromethane
Solvent volume	250 mL	190 – 220 mL
Temperature	80 °C	150 °C
Pressure	Atmospheric	1500 psi
Time	16 hours	20 minutes
Analytical	HRGC-HRMS	HRGC-HRMS

Clean Up Procedures

Following extraction, solvent was reduced to incipient dryness, prior to reconstitution in *ca* 1 ml hexane and purification through a silica gel impregnated with sulphuric acid column and then treated with florisil.

Analytical Procedures

Standards of both the isotopically labelled and native 2378 containing congeners of interest are injected sequentially, starting with the least concentrated.

As a check upon the efficiency of the extraction/clean up, $^{13}\text{C}_6$ -1,2,3,4-TCDD was added to the samples immediately prior to injection onto the GC/MS system. This is also used to help evaluate the method detection limit in the case where no peak is detected for one of the targeted analytes.

Results and Discussion

Based on the results of several experiments, the conditions presented in Table 2 showed better results for the extraction of CPP. These conditions may be adjusted to optimize extraction efficiency. Obviously this optimization depends on the matrix.

Table 2: Recommended conditions for the extraction of PCDD/F's in CPP samples.

Pressure	1500 psi	Purge	120 seconds
Temperature	150 °C	Flush % volume	30
Heat	5 minutes	Cycles	3
Static cycles	2 minutes	Solvent	Dichloromethane

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The results show that solvent volume values in the ASE method is smaller than the Soxhlet extraction. This is other advantage of this method regarding the analysis costs.

The percentual recovery data are important parameters to compare different methods, manily in extraction procedures. Table 3 shows the recovery results obtained.

Table 3: Recovery data.

Dioxins	Recovery % ASE	Recovery % Soxhlet	Furans	Recovery % ASE	Recovery % Soxhlet
2,3,7,8-TCDD	86	74	2,3,7,8-TCDF	90	78
1,2,3,7,8-PeCDD	105	88	1,2,3,7,8-PeCDF	95	84
1,2,3,6,7,8-HxCDD	86	78	2,3,4,7,8-PeCDF	99	87
1,2,3,4,7,8-HxCDD	115	94	1,2,3,4,7,8-HxCDF	136	89
1,2,3,7,8,9-HxCDD	118	101	1,2,3,6,7,8-HxCDF	90	83
1,2,3,4,6,7,8-HpCDD	123	104	2,3,4,6,7,8-HxCDF	105	90
OCDD	128	105	1,2,3,7,8,9-HxCDF	132	106
			1,2,3,4,6,7,8-HpCDF	106	92
			1,2,3,4,7,8,9-HpCDF	127	108
			OCDF	135	112

Tables 4 and 5 present the results of the dioxins and furans of the spiked CPP analysis. These results show that ASE method is a great promise and suitable method for this analysis.

Table 4: Results for Accelerated Solvent Extraction Method.

Accelerated Solvent Extraction						
Dioxins	Obtained value	True value	Difference	Difference %	TEF	I-TEQ
2,3,7,8-TCDD	70	83	-13	-16	1	70
1,2,3,7,8-PeCDD	67	83	-16	-19	0.5	33.5
1,2,3,6,7,8-HxCDD	80	83	-3	-4	0.1	8
1,2,3,4,7,8-HxCDD	89	83	6	7	0.1	8.9
1,2,3,7,8,9-HxCDD	65	83	-18	-22	0.1	6.5
1,2,3,4,6,7,8-HpCDD	90	83	7	8	0.01	0.9
OCDD	105	83	22	26	0.001	0.105
Furans						
2,3,7,8-TCDF	100	83	17	20	0.1	10
1,2,3,7,8-PeCDF	84	83	1	1	0.05	4.2
2,3,4,7,8-PeCDF	95	83	12	14	0.5	47.5
1,2,3,4,7,8-HxCDF	88	83	5	6	0.1	8.8
1,2,3,6,7,8-HxCDF	81	83	-2	-2	0.1	8.1
2,3,4,6,7,8-HxCDF	74	83	-9	-11	0.1	7.4
1,2,3,7,8,9-HxCDF	59	83	-24	-29	0.1	5.9
1,2,3,4,6,7,8-HpCDF	98	83	15	18	0.01	0.98
1,2,3,4,7,8,9-HpCDF	58	83	-25	-30	0.01	0.58
OCDF	106	83	23	27	0.001	0.106
Grand Total TEQ	222	240	-----	- 8.0	-----	-----

Table 5: Results for Soxhlet Extraction Method.

Soxhlet Extraction						
Dioxins	Obtained value	True value	Difference	Difference %	TEF	I-TEQ
2,3,7,8-TCDD	75	83	-8	-10	1	75
1,2,3,7,8-PeCDD	79	83	-4	-5	0.5	39,5
1,2,3,6,7,8-HxCDD	74	83	-9	-11	0.1	7,4
1,2,3,4,7,8-HxCDD	79	83	-4	-5	0.1	7,9
1,2,3,7,8,9-HxCDD	73	83	-10	-12	0.1	7,3
1,2,3,4,6,7,8-HpCDD	140	83	57	69	0.01	1,4
OCDD	510	83	427	514	0.001	0,51
Furans						
2,3,7,8-TCDF	94	83	11	13	0.1	9,4
1,2,3,7,8-PeCDF	99	83	16	19	0.05	4,95
2,3,4,7,8-PeCDF	99	83	16	19	0.5	49,5
1,2,3,4,7,8-HxCDF	84	83	1	1	0.1	8,4
1,2,3,6,7,8-HxCDF	88	83	5	6	0.1	8,8
2,3,4,6,7,8-HxCDF	86	83	3	4	0.1	8,6
1,2,3,7,8,9-HxCDF	72	83	-11	-13	0.1	7,2
1,2,3,4,6,7,8-HpCDF	160	83	77	93	0.01	1,6
1,2,3,4,7,8,9-HpCDF	86	83	3	4	0.01	0,86
OCDF	420	83	337	406	0.001	0,42
Grand Total TEQ	240	240	-----	0.0	-----	-----

Conclusions

The data presented show that the Accelerated Solvent Extraction is essentially equivalent to conventional extraction technique of dioxins and furans analysis for the CPP samples. In addition to being equivalent, ASE can be performed reducing a operational time for the extraction step and with much less solvent volume values in comparison to Soxhlet extraction.

References

1. G.K. Carvalhaes *et al.* Lime as the Source PCDD/F contamination in citrus pulp pellets from Brazil, *OrganoHalogen compounds* 41 , p.137 , (1999)
2. G.K. Carvalhaes *et al.* PCDD/F contamination in citrus pulp pellets from Brasil: Status of the monitoring program, *OrganoHalogen compounds* 46 , p.260 , (2000)