COMPARISON BETWEEN LLE AND LSE FOR EXTRACTING DIOXINS IN MILK

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Introduction

It is in the continuous monitoring process based on the detection as well as clean up and extraction in order to measure the levels of dioxin-like compounds that has been grouped into endocrine disruptors because of similar biological process and structures.[1-2] It is needed more effective extracting process especially when liquid liquid extraction (LLE) is widely used in the analysis of dioxins in liquid food samples.[3] To optimize analytical method on the process of extracting 2,3,7,8-chlorinated PCDDs (7 congeners), PCDFs (10 congeners) and non-ortho co-planar PCBs (#77, #81, #126, #169) in milk, LLE and liquid solid extraction (LSE) were compared.

Methods and Materials

Milk samples are spiked with the stable isotope labelled($^{13}C_{12}$) analogs before extraction. For LLE, milk (50g), sodium oxalate (0.5g) and ethanol (50ml) was added into a teflon bottle (500ml) and then extracted with hexane:ether (1:1, 100ml) for 15 minutes by wrist-action shaker, three times. The combined organic layers were concentrated under the reduced pressure. For LSE, milk was digested with acetonitrile:water (1:1, 20ml) and sodium oxalate (0.2g) in a teflon bottle(500ml) by wrist-action shaker for 30 minutes. It was loaded onto the solid phase, C18 cartridge (10g, 75ml) at 5ml/min, which was pre-activated with water (10ml) and methanol (100ml) at 20ml/min by the peristaltic pump. After rinsing the cartridge with water (10ml) and methanol (2ml), it was dried completely under the vacuum (10psi) for 1.5 hours and eluted with hexane (12ml, three times) at 5ml/min as shown in figure 1. After extracting samples either by LLE or by LSE, the resulting extracts were cleaned up and then analyzed by high resolution gas chromatography/high resolution mass spectrometry based on the process of KFDA.[4-7]

Results and Discussion

As results the recovery of LLE was 89.5~173.0% for TCDD, 102.1~103.3% for PeCDD, 85.0~117.1% for HxCDD, 98.0~104.0% for HpCDD, 87.8~112.6% for OCDD, 102.5~135.0% for TCDF, 106.6~121.5% for PeCDF, 78.4~126.9% for HxCDF, 105.9~108.8% for HpCDF, 78.8~100.2% for OCDF, 103.6~107.9% for 33'44'-TCB, 90.4~95.8% for 344'5-TCB, 105.3~111.7% for 33'44'5-PeCB, 98.8 ~109.0% for 33'44'55'-HxCB, respectively.

The recovery of LSE was 85.2~132.0% for TCDD, 95.1~105.1% for PeCDD, 74.6~93.6% for HxCDD, 91.6~92.0% for HpCDD, 93.6~101.1% for OCDD, 85.3~96.9% for TCDF, 96.7~102.2% for PeCDF, 88.8~97.8% for HxCDF, 83.4~99.0% for HpCDF, 86.8~93.3% for OCDF, 125.0~127.7% for 33'44'-TCB, 103.2~108.3% for 344'5-TCB, 75.9~112.9% for 33'44'5-PeCB, 113.2 ~114.8% for 33'44'55'-HxCB, respectively. In addition, the average recovery was 89~128% for LLE and 75~126% for LSE. The reproducibility determined in three independent series showed a CV varying from 0.58% to 32.8% for LLE and from 0.22% to 21.66% for LSE. The spiked and calculated amount for LLE & LSE are shown in Table 1 and there is no big difference between LLE and LSE. However, in case of performing LLE, much of the time has been spent for separating the organic phase from the aqueous phase as well as it is very laborious, whereas LSE is rapid and simple. Therefore, LSE is a promising method for analyzing the dioxin-like compounds in milk.

References

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Table 1. Comparison of the Extraction efficiency of Dioxin-like compounds in Milk using LLE and LSE

(n=3)

Compounds	Spiked amount (pg/μL)	Calculated amount (pg/μL)±SD	
		LLE	LSE
2378-TCDD	2.00	2.56±0.84	2.17±0.47
12378-PeCDD	10.00	10.26±0.06	10.01±0.50
123478-HxCDD	10.00	10.77±0.71	7.52±0.06
123678-HxCDD	10.00	9.04±0.47	9.07±0.29
123789-HxCDD	10.00	9.95±1.53	9.00±0.39
1234678-HpCDD	10.00	10.01±0.34	9.18±0.02
OCDD	20.00	20.59±2.67	19.47±0.75
2378-TCDF	2.00	2.32±0.34	1.83±0.12
12378-PeCDF	10.00	11.00±0.30	9.98±0.28
23478-PeCDF	10.00	11.51±0.57	10.05±0.10
123478-HxCDF	10.00	10.70±0.26	9.50±0.25
123678-HxCDF	10.00	10.23±2.07	9.19±0.34
234678-HxCDF	10.00	12.31±0.34	9.54±0.17
123789-HxCDF	10.00	9.79±0.46	8.98±0.11
1234678-HpCDF	10.00	10.70±0.16	9.53±0.47
1234789-HpCDF	10.00	9.83±1.67	8.63±0.27
OCDF	20.00	17.73±2.15	17.80±0.73
344'5-TCB	10.00	9.26±0.29	10.63±0.27
33'44'-TCB	10.00	10.53±0.23	12.61±0.14
33'44'5-PeCB	10.00	10.92±0.34	9.95±2.05
33'44'55'-HxCB	10.00	10.31±0.53	11.41±0.08

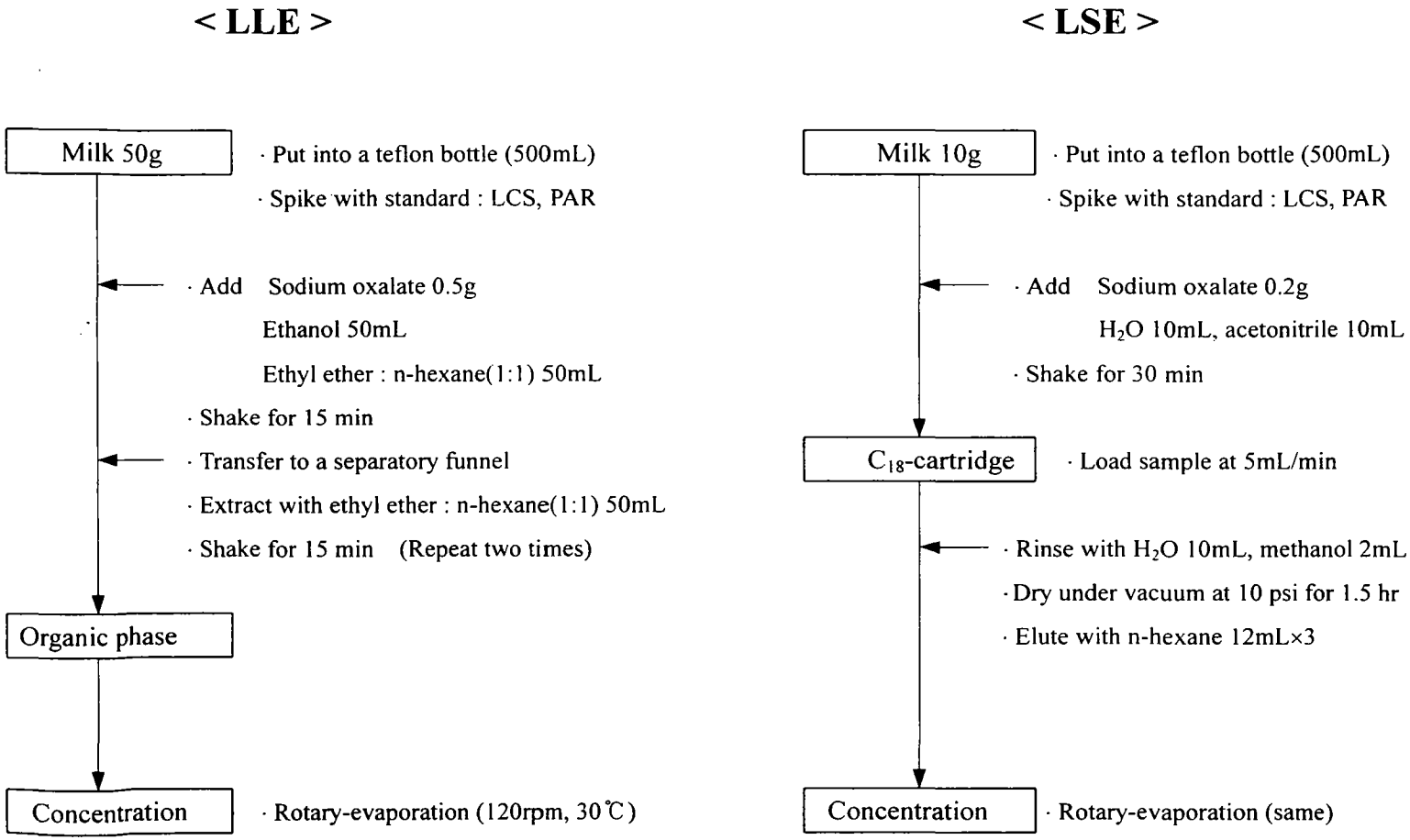

Fig. 1. Schematic diagram for extraction