COMPARISON OF SOXHLET AND ACCELERATED SOLVENT EXTRACTIONS IN THE ANALYSIS OF DIOXINS AND FURANS FROM LIVER SAMPLES

Janice K. Huwe

USDA, ARS, Biosciences Research Laboratory, P.O. Box 5674, Fargo, ND, 58105, USA

Introduction

Accelerated solvent extraction (ASE) has several benefits over conventional Soxhlet extraction including reduced solvent consumption and decreased extraction times. These benefits have made ASE an encouraging substitute for Soxhlet extractions in a number of pesticide and food extraction applications. ASE has been officially accepted by the U.S. EPA as a technique for the extraction of polychlorinated biphenyls (PCBs), polychlorinated dioxins and furans (PCDD/Fs), semivolatiles, and certain pesticides from sediments and sludge (U.S. EPA Method 3545A). ASE has been shown to perform as well as or better than Soxhlet in the extraction of PCDD/Fs from fly ash,[1] soils and sediments,[2,3] and citrus pulp.[4] Recoveries were equivalent or higher than Soxhlet methods, and extraction times decreased from over 16 h to under 1 h.

When applied to biological samples, extraction techniques must not only be validated for the recovery of the analytes but also the associated lipids, if quantitation is to be made on a lipid-weight basis. ASE has been demonstrated to be similar to Soxhlet extraction in the recoveries of lipids and organohalogen compounds from eggs[5] and dry or wet fish homogenates.[6,7] In our laboratory, many studies are focused on the disposition of PCDD/Fs into animal tissues and organ compartments. We have therefore conducted a study to compare the use of ASE and Soxhlet extraction in the analysis of PCDD/Fs from beef liver.

Methods and Materials

The liver tissue analyzed in this study contained incurred levels of PCDD/Fs from a previously reported feeding study in cattle.[8] The whole liver from individual cattle was homogenized prior to sampling. For Soxhlet extraction, a typical extraction for dioxins described in EPA Method 8290A was employed. Briefly, a 10 g sample was ground with approximately 100 g dry Na_2SO_4, spiked with ^{13}C-labeled PCDD/F surrogates (Wellington Labs), and extracted 20 h with 300 ml hexane/methylene chloride (1:1). For accelerated solvent extraction, a 10 g sample was ground with approximately 10 g Celite, spiked with ^{13}C-labeled PCDD/F surrogates, packed into an extraction cell using Ottawa sand to fill any void, and extracted on a Dionex ASE 200 Accelerated Solvent Extractor using the methods listed in Table 1. After ASE, the organic extracts were dried with Na_2SO_4 (5 g) and filtered through glass wool.

An aliquot of each organic extract was evaporated to dryness, and lipid weights were determined gravimetrically. The remainder of the extract was further purified according to EPA Method 8290 using sequential extractions with 20 % KOH and concentrated H_2SO_4 followed by chromatography on acidic, basic, and neutral silica gel, basic alumina, and carbon columns. PCDD/Fs were quantitated by high resolution GC-high resolution MS. Each extraction method was repeated in triplicate. Soxhlet 1 and ASE methods A-C were performed with one liver; Soxhlet 2 and ASE methods D–E were performed with a second liver.

Table 1. Selected parameters for the ASE methods tested in this study. Each method had a 5 min static time and three static cycles.

ASE Method	Pressure (psi)	Temp. (°C)	Heat time (min)	Solvents
A	1500	100	5	Hexane/methylene chloride (1:1)
B	2000	150	7	Toluene
C	1000	125	6	Hexane/iso-propanol (3:2)
D	1500	125	6	Methylene chloride/iso-propanol (3:2)
E	1000	125	6	Hexane/methylene chloride/iso-propanol (1:1:1)

Results and Discussion

The initial ASE methods evaluated were based on methods that had been applied to PCDD/F extractions from eggs[5] (method A) or solids[1-4] (method B). Table 2 shows that while recoveries of PCDD/Fs were acceptable with method A (55–85 %), the lipid recovery was approximately half that of the lipid recovered from the Soxhlet extraction. Method B, using toluene as an extraction solvent, proved to be an unsuccessful method for liver tissue. Recoveries of the PCDD/Fs were low (34 % on average), and the percent lipid recovered was 40 % lower than the value determined by Soxhlet. In addition, a white solid precipitated from the toluene extract making filtration and further purification steps difficult.

Methods C–E included iso-propanol as a co-solvent. Method C is an ASE method developed for the extraction of fats from food products (Dionex technical bulletin AN321) and gave excellent recovery of lipids from the liver samples. However, recovery of the PCDD/Fs averaged less than 33%. Both methods D and E gave somewhat better recoveries of the lipids than the Soxhlet method, 120% and 114%, respectively. These ASE methods also had average recoveries of over 70 % for the PCDD/Fs.

Table 3 shows the quantitation of PCDD/Fs for the Sohxlet extraction and each of the five ASE methods. On a wet weight basis, the total toxic equivalency (TEQ) for the ASE methods varied from 80–111 % of the Soxhlet values, within the accepted limits of EPA Method 8290A for dioxin analysis. The relative standard deviations were similar to on-going precision measurements for dioxins at our laboratory (5–12 %). Lipid-adjusted TEQs, however, ranged from 84–181 % of the Soxhlet values and relative standard deviations reflected the higher variability in the calculated lipid percent (up to 24 %).

Overall, ASE methods D and E appeared to be the best extractions for PCDD/Fs and lipids from liver. Recoveries of PCDD/Fs were between 57 and 94 % while the percent lipid recovered was 114–120 % of the amount recovered from Soxhlet. The use of a polar co-solvent (iso-propanol) may be more effective in penetrating and dissolving residual water in the liver tissue and make recovery of the

Table 2. Percent recoveries of PCDD/Fs and percent lipids calculated for Soxhlet and ASE methods. Recoveries of PCDD/Fs are based on the ^{13}C-PCDD/F surrogates. The range represents individual congener values, the average represents all congeners and is based on three replicates. Relative standard deviations (%) are in parentheses.

	Soxhlet 1	ASE A	ASE B	ASE C	Soxhlet 2	ASE D	ASE E
Recovery Range	55.1 – 78.1	55.1 – 84.7	27.6 – 46.3	23.2 – 42.9	44.4 – 87.7	60.5 – 86.9	57.4 – 93.7
Av. Recovery	66.2 (4.0)	73.5 (11.3)	34.0 (9.2)	32.8 (16)	67.1 (17)	72.2 (2.9)	73.2 (8.9)
% lipid	4.35 (5.0)	2.12 (8.6)	2.69 (11)	4.48 (3.1)	3.12 (21)	3.75 (24)	3.56 (3.9)

Table 3. Lipid adjusted concentrations of 17 toxic PCDD/Fs and lipid adjusted and wet weight toxic equivalencies (TEQ) compared for Soxhlet and ASE methods (pg/g). Values are averages of three replicates. Relative standard deviations (%) are in parentheses. nd = not detected.

Congener	Soxhlet 1	ASE A	ASE B	ASE C	Soxhlet 2	ASE D	ASE E
2,3,7,8TD	202 (6.0)	371 (16)	278 (6.3)	165 (1.5)	255 (22)	229 (17)	263 (7.8)
1,2,3,7,8PeD	680 (4.4)	1172 (15)	821 (6.5)	537 (2.4)	938 (22)	883 (19)	843 (8.8)
1,2,3,4,7,8HxD	658 (6.8)	1002 (14)	689 (6.9)	416 (4.0)	871 (21)	901 (20)	874 (2.4)
1,2,3,6,7,8HxD	2399 (6.6)	4132 (13)	2962 (3.1)	1879 (2.0)	5255 (20)	4819 (24)	4854 (3.6)
1,2,3,7,8,9HxD	766 (6.2)	1211 (15)	864 (8.3)	475 (3.0)	1255 (27)	1225 (20)	1144 (9.6)
1,2,3,4,6,7,8HpD	64462 (2.8)	119371 (17)	83446 (4.8)	56088 (1.3)	136384 (23)	125604 (19)	129964 (7.4)
OCDD	188746 (6.1)	345711 (16)	250498 (7.1)	173289 (2.6)	408520 (26)	384514 (21)	399348 (2.9)
2,3,7,8TF	2.4 (173)	13.1 (5.7)	4.2 (87)	2.5 (93)	7.2 (21)	5.8 (95)	nd
1,2,3,7,8PeF	nd	1.1 (109)	nd	nd	nd	nd	1.0 (173)
2,3,4,7,8PeF	806 (2.0)	1548 (14)	1120 (8.7)	712 (0.7)	1232 (23)	1137 (24)	1210 (4.3)
1,2,3,4,7,8HxF	161 (3.7)	333 (13)	247 (4.8)	161 (3.1)	547 (24)	526 (23)	530 (2.8)
1,2,3,6,7,8HxF	78 (7.1)	160 (17)	122 (13)	80 (0.4)	230 (15)	222 (20)	217 (6.7)
2,3,4,6,7,8HxF	121 (2.5)	245 (11)	173 (14)	114 (5.4)	341 (26)	324 (22)	340 (8.5)
1.2.3.7.8.9HxF	nd	1.5 (61)	0.2 (173)	0.5 (43)	nd	2.7 (173)	nd
1,2,3,4,6,7,8HpF	4231 (2.9)	7740 (17)	5428 (6.1)	3612 (1.5)	9160 (21)	8756 (22)	8735 (5.0)
1,2,3,4,7,8,9HpF	232 (4.4)	458 (19)	320 (7.9)	208(5.0)	551 (25)	546 (14)	600 (5.7)
OCDF	7524 (3.2)	14877 (17)	9876 (8.8)	6493 (3.0)	12798 (26)	11684 (22)	12456 (3.8)
TEQ lipid weight	2249 (3.7)	4078 (15)	2907 (6.0)	1881 (1.2)	4163 (22)	3873 (20)	3941 (5.4)
TEQ wet weight	97.8 (4.4)	85.7 (6.1)	78.0 (6.2)	84.3 (4.2)	126 (1.7)	140 (5.9)	140 (1.8)

analytes more facile. Iso-propanol may also be a better solvent for polar lipids, such as phospholipids, present in the liver. The addition of methylene chloride, a more polar solvent than hexane, also appeared to improve recoveries of the PCDD/Fs. Compared to Soxhlet extraction, the ASE method decreased extractions times from 20 h to less than 30 min and reduced the amount of solvent by 100 ml per sample. Further optimization of the ASE method may decrease solvent usage even further.

Acknowledgements

The authors would like to acknowledge the technical assistance of Joyce Wold, Montgomary Botschner, Kristin McDonald, and Jean Picard in the preparation of dioxin samples for analysis.

References

1. Windal, I., Miller, D. J., De Pauw, E., and Hawthorne, S. B. (2000) Anal. Chem. 72, 3916–3921.
2. Ryu, I. C., Uom, J. H., Lee, Y. G., Eom, S. W., and Shin J. Y. (2000) Organohalogen Cpds. 45, 78–80.
3. Henkelmann, B., Wottgen, T., Chen, G., Schramm, K. W., and Kettrup, A. (1999) Organohalogen Cpds. 40, 133–136.
4. Carvalhaes, G. K., Brooks, P., Marques, C. G., Azevedo, G. C., Azevedo, J. A. T., and Machado, M. C. S. (2001) Organohalogen Cpds. 50, 225–228.
5. Davis, M., Paper, J., Shafiei, H., and Stanley, J. (1998) Organohalogen Cpds. 35, 105–108.
6. Richter B. E. and Covino, L. (2000) LCGC 18, 1068–1073.
7. Weichbrodt, M., Vetter, W., and Luckas B. (1999) Organohalogen Cpds. 40, 309–312.
8. Feil, V. J., Huwe, J. K., Zaylskie, R. G., Davison, K. L., Anderson, V. L., Marchello, M., and Tiernan, T. O. (2000) J. Agric. Food Chem. 48, 6163–6173.