Sample Pretreatment with Microwave-Assisted Techniques

Zhanxia ZHANG[†], Guohua XIONG, Gongke LIE, and Xiaoqing HE

Department of Chemistry, Zhongshan University, Guangzhou 510275, China

A sample pretreatment by microwave-assisted extraction (MAE), microwave-assisted alkaline degradation (MAAD) and microwave-assisted saponification prior to the determination of PAHs, PCBs, triazines and cholesterol in diverse samples using GC, GC-MS and HPLC has been developed. When MAAD was coupled with MAE, the degradation of chlorinated pesticides and the extraction of PCBs analytes in the samples could be carried out simultaneously. Likewise, when MAAD and MAS were coupled with MAE, the degradation, saponification and extraction could be carried out simultaneously. In addition, a microwave-assisted derivatization (MAD) of fatty acids in edible oil, plasma and shark cartilage has been developed. The analytical results are satisfactory. The microwave-assisted sample pretreatment methods are rapid, labor and solvent saving, moreover, non-chlorinated organic solvents can be used.

(Received October 12, 1999; Accepted November 20, 1999)

Traditional methods of sample preparation for organic analytes, such as Soxhlet extraction and sonication, are both time-consuming and create large amounts of solvent waste. Typical extractions take up to 6 h to complete, and use well over 100 ml of chlorinated organic solvent. Although newly developed supercritical fluid extraction (SFE) has enjoyed much success owing to its organic solvent-free extraction using non-toxic CO₂ as the supercritical solvent, its extraction results depend not only on the solute, but also on the sample matrix. Moreover, more polar hazardous fluids are often required to combine with the organic modifiers to effect complete extraction. In many analytical laboratories, the microwave-assisted dissolution technique is already the method of choice for sample preparation. Other microwave-assisted techniques for sample preparation are being constantly developed and put into practice. The application of microwave energy for the isolation of organic analytes from diverse samples began in the mid-eighties, and has made great progress with the advent of commercial microwave systems.¹ Compared with the traditional sample preparation methods, microwave-assisted extraction (MAE) is rapid (generally 5–10 min), solvent saving (generally 20–30 ml of non-chlorinated organic solvent.) and efficient.

Recently, we have developed a series of microwave-assisted techniques for sample pretreatment prior to GC, GC-MS or HPLC determination of target organic analytes in diverse samples. The microwave-assisted experiments were performed with a 600 W microwave sample preparation system (Model MK-1, Xin-Ke Institute of Applied Microwave Technology, Shanghai, China). The operating conditions could be optimized by mainly adjusting three parameters, namely, the magnetron power output, usually set at 100%, the irradiation time (or heating time) and the controlled pressure value. The pressure in the vessel could be indicated by a special-designed optical fiber sensor. There were eight separate stages for pressure selection,

and a pressure as high as 4 MPa could be attained. The volume of the PTFE lined digestion vessel was about 60 ml. The turntable could hold nine vessels simultaneously.

GC determinations of PAHs, PCBs and triazines were performed on a HP-5 column (0.25 mm, 0.25 µm phase thickness) using a Hewlett-Packard 4890D series gas chromatograph equipped with an electron-capture detector (ECD), a nitrogen phosphorus detector (NPD) and a split/splitless injector operated in the splitless mode. The chromatograph data were obtained and processed in a HP Chemstation (G2070AA, A.04.01).

A HP6890 series gas chromatography interfaced to a HP 5973 mass-selective detector with a HP MS Chemstation data system (G1701AA, B.02.05) was used for MS identification of the GC peaks. The column used was a HP-5MS cross-linked fused-silica capillary column (30 m × 0.25 mm, 0.25 µm, phase thickness) coated with 5% phenyl-polymethylsiloxane. The HPLC used for the analyses of triazines was a Shimadzu LC-4A with an SPD-1 UV-VIS detector.

Microwave-assisted extraction

The microwave-assisted extraction (MAE) of three types of common organic pollutants, namely polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs) and triazine herbicides from soils, sediments and mussels, were studied.^{2–5} The operating parameters of the microwave system for each type of pollutants in various matrices were optimized by using a three-level orthogonal array design. The experimental results show that high MAE efficiencies could be obtained if the magnetron power output was set at 100% (600 W), the pressure at the lowest stage (0.1–0.5 MPa) and the irradiation time 4–6 min for a 1–2 g sample. The MAE efficiency was also influenced by the solvent type used. Various solvents (or extractants), namely methanol, ethanol, KOH-methanol, KOH-ethanol, acetone-hexane, dichloromethane, water, and surfactants have been tried. Table 1 lists the solvents that yielded recoveries of the target analytes well above 80% with 1 mol/L KOH-methanol, methanol, acetone-hexane, water and surfactants. This indicates that chlorinated organic solvents could be left off. It is shown below that 1 mol/L KOH methanol

[†] To whom correspondence should be addressed.

This paper was presented at ASIANALYSIS V, Xiamen, China, May 4–7, 1999.

Table 1 Effect of the solvent type on the MAE efficiency

Analyte	Recoveries (%) of analytes with various solvents					
	Methanol	1 mol/L KOH-methanol	Acetone-hexane	Water	Triton X-100 (0.5%)	CTAB (0.5%)
Atrazine	99.8	—	90.4	91.5	—	—
Simazine	100.4	—	92.7	88.5	—	—
Prometryne	97.3	—	86.3	87.6	—	—
PCB 1248	86.3	83.8	89.2	—	—	—
PCB 1242	84.2	82.8	87.5	—	—	—
Naphthalene	—	—	79.5	—	88.6	84.5
Acenaphthylene	—	—	89.7	—	84.6	82.4
Fluorene	—	—	91.5	—	84.3	83.4
Anthrene	—	—	86.1	—	87.3	84.5
Chrysene	—	—	91.2	—	81.4	80.2
Benzo[a]pyrene	—	—	90.6	—	82.1	80.5

“—” indicates no experiment was performed. 30 mL solvent was used for 1-g sample. The magnetron power output was set at 100%; the MAE pressure set at first stage (0.1 – 0.5 MPa); the irradiation time 5 – 6 min.

Table 2 Effect of soil matrices and moisture on extraction efficiencies of analytes by MAE

Analyte	Extraction efficiency, %					
	Sandy loam	Rich clay	Poor clay	Florisil	Soil moisture, %	Soil moisture, %
Atrazine	—	95.4	98.9	99.2	89.3-	91.5--
Simazine	—	96.3	99.5	99.8	90.7	91.6
Prometryne	—	92.5	98.6	99.1	78.0	85.5
PCB 1248	86.3	83.9	90.1	96.0	90.5	89.0
PCB 1242	84.2	83.1	90.5	96.7	89.6	88.8
Naphthalene	79.5	85.0	85.5	84.8	81.0	82.0
Acenaphthylene	89.7	94.2	95.6	95.8-	89.1	90.8
Fluorene	91.5	93.4	94.7	94.0	92.6	93.7
Anthrene	86.1	89.1	88.7	89.5	85.4	87.0
Chrysene	91.2	94.3	97.8	96.2	92.0	93.5
Benzo[a]pyrene	90.5	95.1	97.3	95.5	92.1	91.7

“—” indicates no experiment was performed. 30 mL acetone-hexane (1:1, v/v) for 1-g sample. The magnetron power output set at 100%; the MAE pressure set at first stage (0.1 – 0.5 MPa); irradiation time 5 – 6 min. Sandy loam contains ~1% organic carbon, rich clay ~0 – 0.5% organic carbon.

Table 3 Comparison of recoveries of PAHs in soils obtained by MAE and Soxhlet extraction

PAH	Ave. recovery, %		
	MAE for 6 min	Soxhlet for 1.5 h	Soxhlet for 4 h
Nephthalene	79.5	72.6	77.6
Acenaphthylene	89.7	82.1	91.5
Fluorene	91.5	84.7	92.8
Phenanthrene	87.4	81.4	88.0
Anthrene	86.1	79.6	84.5
Chrysene	91.2	83.8	92.4
Benzo[a]pyrene	90.5	82.5	91.0

MAE: 30 mL acetone-hexane (1:1, v/v) for 1-g sample; magnetron power output set at 100%; pressure set at first stage (0.1 – 0.5 MPa); irradiation time 5 – 6 min.

Soxhlet E: 100 mL acetone-hexane (1:1, v/v) for 1-g sample; two heating time: 1.5 h and 4 h.

certainly efficient for MAE; *e.g.* dichloromethane, due to its poor ability to absorb microwave energy, is not so efficient as methanol or even as water to extract triazines. Water used as an extractant in MAE is not only friendly to the environment, but also very easy to couple with ELISA analysis.² Table 1 also indicates that a surfactant is an efficient solvent for the extraction of PAHs from soils. Since the concentration of the surfactant used was about 1.0%, a surfactant is on the whole cheaper, less toxic and safer compared with other organic solvents.

The experimental results also indicated that the sample matrix and moisture did not exert any significant effects on the recovery of the target analyte. As can be seen from Table 2, the level of organic carbon in the soils could influence the extraction efficiency of PCBs in soils; *i.e.*, the higher the organic carbon content the lower the extraction efficiency. This may have been due to the absorption affinity of PCBs to the organic carbon in the soil. However, the decrease was only a few percent.

Table 3 gives a the comparison of the extraction efficiencies of PAHs in soils by MAE and by Soxhlet extraction. It can be seen that a much longer extraction time is needed to extract PAHs with Soxhlet extraction (4 h) than with MAE (6 min) to obtain the appropriate extraction efficiencies. After MAE, the vessel was cooled down to room temperature; then, the

is not only an efficient extraction solvent for PCBs, but also a very useful solvent for the alkaline degradation of interferences caused by chlorinated pesticides. It is worth noticing that the solvents generally efficient for Soxhlet extraction are not

Table 4 Comparison of alkaline degradation efficiencies of chlorinated pesticides obtained by MAAD and centrifuging alkaline degradation

Degradation method	Alkaline degradation efficiency, %									
	α -HCH	β -HCH	γ -HCH	δ -HCH	Aldrin	Dieldrin	<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	<i>p,p</i> -DDT	<i>p,p'</i> -DDT
MAAD	100	100	100	100	55	62	-43	100	100	100
MAAD*	100	100	100	100	65	100	-43	100	100	100
Cen. M	100	92	100	100	34	21	-80	100	100	100

MAAD: microwave-assisted alkaline degradation with 30 mL 1 mol/L KOH-methanol for 4 min. MAAD*: microwave-assisted alkaline degradation with 30 mL 1 mol/L KOH-methanol for 4 min. Then the degradation products purified with H₂SO₄. Cen. M: centrifuging alkaline degradation with 10 mL 1 mol/L KOH-methanol centrifuged at 3500 rpm for 30 min.

supernatant from each vessel was carefully decanted and used directly for chromatographic analysis.

Microwave-assisted alkaline degradation

PCBs in environmental samples are generally determined with a highly sensitive GC method after sample pretreatment. However, the coexisting chlorinated pesticides (CPs) usually present interference. The column techniques are generally used for the separation of CPs from PCBs. For example, Berset *et al.*⁶ used a double-layer column consisting of silica gel in the bottom and aluminum oxide in upper layers to separate the CPs from PCBs in soil, liquid manure and sewage sludge. Fuoco *et al.*⁷ used a Florisil column and Bernal *et al.*⁸ used sulfuric acid and a Florisil clean-up procedure to determine PCBs in water and reference materials, respectively. However, these methods are laborious and consume a large amount of organic solvents.

We have developed a microwave-assisted alkaline degradation (MAAD) method using 1 M KOH-methanol as a solvent for the degradation of CPs in soil.⁹ The experimental results showed that under optimized conditions, the interferences of CPs on PCBs in soil could be eliminated, as shown in Table 4. The data showed that α -HCH, β -HCH, γ -HCH, δ -HCH, *o,p*-DDT, *p,p'*-DDD and *p,p'*-DDT could be degraded completely, and *p,p'*-DDE, Aldrin and Dieldrin partly. The “-” sign indicates that new chromatographic peaks appeared and overlapped on the original peak after MAAD. However, these MAAD products of Aldrin and Dieldrin in the solution could be completely eliminated by rinsing it with concentrated sulfuric acid after MAAD. The mean recovery of PCBs obtained was 85.3% with a RSD of 3.0%. The experimental results also indicate that the alkaline degradation of the interferences and the extraction of the target PCBs could be carried out simultaneously, using 1 mol/L KOH-methanol.

We compared the efficiency of MAAD with that of the centrifuging alkaline degradation method. The results are presented in Table 4. It can be seen that the alkaline degradation rates with centrifuging for β -HCH, Aldrin and Dieldrin were 91.6%, 33.8% and 21.3%, respectively, while those with MAAD were 100%, 55.3% and 61.6%, respectively. The degradation for the other pesticides was 100% with either method. However, the main advantage of MAAD over the centrifuging method is that it can be carried out simultaneously with MAE of PCBs, while the latter can only be carried out after PCBs extraction. Moreover, since MAAD can be operated at a higher temperature, the degradation time can be saved from 30 to 6 min. To avoid the emulsion formed during the MAAD and MAE procedures, a 5% Na₂SO₄ solution was added to the extract. Thus, after MAE and MAAD, the soil sample is extracted with hexane, then rinsed with concentrated H₂SO₄ and 5% NaCl solution, respectively. After drying with dehydrated Na₂SO₄, the sample is ready for GC-ECD determination of PCBs. In addition,

no further cleaning with Al₂O₃-SiO₂ column is necessary.

Microwave-assisted saponification

Saponification is generally employed for the pretreatment of biological samples prior to the determination of organic analytes. This is not only for the isolation of fats from organic tissues, but also for removing sulfur and degrading some chlorinated pesticides. Conventionally, the samples are immersed in a KOH-ethanol (or NaOH-ethanol, or KOH-methanol) solution and refluxed in a water-bath for about 1 h. However, Van der Valk *et al.*¹⁰ found that some degradation of PCBs would occur when sample saponified at 90°C.

We have developed microwave-assisted saponification (MAS) methods for the mussel and serum samples pretreatment. It was found that 15 g mussel could be completely saponified within 2 min with a 30 mL 1 mol/L KOH-methanol solution.¹¹ Experimental results also indicate that the chlorinated pesticides (CPs), generally coexisting with PCBs in mussel, were almost completely degraded simultaneously, whereas no detectable degradation of PCB1242 and PCB1248 was observed. In addition, no further cleaning with an Al₂O₃-SiO₂ column was necessary after saponification. The results are satisfactory.

MAS has also been used for the saponification of a serum sample prior to the determination of cholesterol by GC.¹² The saponification time for 0.5 ml serum is only 30 s with 5 ml 0.4 mol/L KOH-methanol and 1 mL heptane, whereas the conventional water-bath reflux method needs almost 1 h. Moreover, the microwave-assisted extraction of cholesterol with heptane can be carried out simultaneously with saponification.

Microwave-assisted derivatization

The use of microwave-assisted derivatization (MAD) for sample pretreatment in organic environmental analysis was first reported by Lalere *et al.*¹³ Recently, we also developed a series of MAD methods for the derivatization of fatty acids in edible oil,¹⁴ plasma¹⁵ and shark cartilage¹⁶ samples prior to their GC-MS analysis. The derivatization of fatty acids in edible oil was carried out with 0.4 mol/L KOH-methanol or methanol-toluene-H₂SO₄ in a closed vessel with 1 min of microwave irradiation (600 W). The esterified product was then extracted with heptane plus 5% NaCl. The extract could be determined directly with GC-MS. The microwave-assisted digestion and MAD of fatty acids in plasma was also carried out with methanol-toluene-H₂SO₄ in a closed vessel, but with only 40 s of microwave irradiation (600 W), whereas the digestion and MAD of fatty acids in shark cartilage was carried out with HCl-methanol with 4 min of microwave irradiation. Traditionally, the derivatization of fatty acids usually takes an hour to complete in a hot water-bath using H₂SO₄-methanol or KOH-methanol, and the esterified product is extracted with toxic benzene-petroleum-ether. Thus, compared with the traditional derivatization procedure, the

Table 5 Comparison of analytical results of fatty acids obtained by MAD and traditional derivatization method (hot water bath)

Carbon number	Relative concentration of fatty acids, %		Total ionic mass peak area ratio
	MAD	Hot water bath	
C _{16:0}	13.30	13.11	2.1
C _{18:2}	37.58	37.49	2.1
C _{18:1} ¹³	38.1	40.58	1.9
C _{18:2} ⁸	4.52	3.69	2.5
C _{18:0}	1.28	0.76	3.5
C _{20:1}	1.68	1.11	3.1
C _{20:0}	2.99	1.93	3.2

MAD developed is rapid, efficient, sensitive and solvent saving, as shown in Table 5. From this table, it can be seen that the relative concentration of fatty acids obtained with these two methods were almost the same, but the ratio of peak area of total ionic mass of target analyte using MAD to that of traditional method was 2–3 times higher. This indicates that the MAD of the sample pretreatment is much more efficient than the traditional method. In addition, the digestion and derivatization of the target analyte can be carried out simultaneously.

References

1. F. E. Smith and E. A. Arsenault, *Talanta*, **1996**, *43*, 1207.
2. G. H. Xiong, J. M. Liang, S. C. Zou, and Z. X. Zhang, *Anal. Chim. Acta*, **1998**, *371*, 97.
3. G. H. Xiong, J. M. Liang, S. C. Zou, X. Q. He, and Z. X. Zhang, *Gaodeng Xuexiao Huaxue Xuebao*, **1998**, *19*, 1560.
4. G. H. Xiong, B. Y. Tang, X. Q. He, M. Q. Zhao, and Z. X. Zhang, *Talanta*, **1999**, *46*, 333.
5. G. H. Xiong, X. Q. He, and Z. X. Zhang, *Anal. Chim. Acta*, submitted.
6. J. D. Berset and R. J. Holzer, *J. Environ. Anal. Chem.*, **1995**, *59*, 145.
7. R. Fucco, M. P. Colombini, and C. Abete, *J. Environ. Anal. Chem.*, **1994**, *55*, 15.
8. J. L. Bernal, M. J. Del Nozal, and J. J. Jimenez, *J. Chromatogr.*, **1992**, *607*, 303.
9. X. Q. He, G. K. Li, G. H. Xiong, and Z. X. Zhang, *Fenxi Huaxue*, **2000**, *26*, 1.
10. V. Van der Valk and Q. T. Dao, *Chempisphere*, **1998**, *17*, 1735.
11. X. Q. He, G. K. Li, Y. Yang, and Z. X. Zhang, *Gaodeng Xuexiao Huaxue Xuebao*, submitted.
12. G. K. Li, X. Q. He, Y. Yang, and Z. X. Zhang, *Fenxi Ceshi Xuebao (J. Instrumental Analysis)*, **1999**, *3*, 15.
13. B. Lalere, J. Szpunar, H. Budzinski, P. Garrigues, O. F. X. Donard, *Analyst*, **120**, 2665 (1995).
14. G. K. Li, X. Q. He, and Z. X. Zhang, *Fenxi Shiyanshi*, **2000**, *21*, 1.
15. G. K. Li, X. Q. He, and Z. X. Zhang, *Fenxi Ceshi Xuebao (J. Instrumental Analysis)*, **2000**, *16*, 1.
16. G. K. Li, X. Q. He, and Z. X. Zhang, *Sepu (Chinese J. Chromatogr.)*, in press.