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MICROWAVE-ASSISTED EXTRACTION: ENVIRONMENTAL APPLICATIONS

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Speed and efficiency are always prime considerations of an analytical technique. In addition, we are seeing environmental considerations – 'greening' of methods – becoming another important factor. A solvent extraction technique that reduces extraction time, improves extraction efficiency and reduces solvent consumption by a factor of 10 is an important one. The latter is critical since it is estimated that about 100 million litres of organic solvent are used annually in organic analytical laboratories worldwide. The use of microwave heating for the extraction of compounds from a variety of sample matrices has been performed since 1985. This review article provides information on the development of the microwave-assisted extraction (MAE) technique, microwave instrumentation and its capabilities, and a perspective for extraction applications.

Development

Microwave heating was first introduced commercially in 1947. It took some time to catch on but it is now a standard item in most kitchens for cooking uses. Its use in the chemistry laboratory has significantly lagged behind domestic applications. Microwaves were first used in 1975 as a heating source for acid digestion under atmospheric conditions. The sample preparation step was reduced from 1–2 h to under 15 min, producing an overall reduction in analysis time. This work initiated the use of microwave energy as a heating source for the chemistry laboratory. Other applications include distillation, organic and inorganic synthesis, evaporation and solvent extraction.

The early applications for MAE were for the extraction of compounds with nutritional interest from plant and animal tissues. From 1986 to 1990, Ganzler and co-workers published a series of four papers exploring the use of microwave energy to partition various compounds from soils, seeds, food and animal feeds as a sample preparation method prior to chromatographic analyses. The extraction step was performed under atmospheric conditions. They extracted a variety of compounds that included antinutritives, crude fat and pesticides. They used solvent schemes similar to their traditional Soxhlet technique to allow a direct comparison of the recoveries between the two techniques. The heating programme consisted of multiple 30 s microwave heating cycles (up to seven cycles) followed by a cooling step. This approach allowed observation of the samples in the microwave cavity to prevent sample boil-over.

The microwave extracts gave recoveries that were 100–120% of the Soxhlet technique. However, the MAE times were a factor of 100 less than the traditional Soxhlet approach. The authors postulated that improved extraction efficiency was due to the polar nature of the extracted compounds or the water contained in the materials. They also noted a decrease in extraction efficiency for the recovery of nonpolar compounds when a nonpolar solvent was used.

The published work of Freitag and John in 1990 expanded the application and technique. These authors explored the use of microwave heating at elevated temperatures and pressure for the extraction of additives from polyolefins. The additives were antioxidants, Irganox 1010 and Irgafos 168, and the light stabilizer Chimassorb 81. The polyolefin matrices were polyethylene and polypropylene with a particle size of 20 mesh and a sample size of 1 g. The MAE was performed with 30 mL of 1,1,1-trichloroethane or a mixture of acetone and *n*-heptane. They obtained 90–100% recoveries of the additives with extraction times of 3–6 min without degradation of the analytes at the elevated temperatures of the extraction. This compared favourably with the conventional 16 h Soxhlet or 0.5–2 h reprecipitation techniques.

Pare *et al.* from the Ministry of Environment of Canada introduced the microwave-assisted process (MAP^{TM}) through a US patent in 1991. In this work, Pare used microwave energy to extract a variety of essential components from natural products and foods, such as biologicals and consumer products. The primary examples are the extraction of essential oils from peppermint, garlic and cedar. The major feature of this work is the release mechanism for the compounds of interest from the substrate. The microwave energy was used to disrupt the glandular and vascular system of the tissue without damaging the surrounding tissue. The solvent was used to trap and dissolve the compounds released from the tissue.

In general, the mechanism involves localized heating of the free water present in the sample. Once the water is at or above its boiling point, the water causes the cell membrane to rupture. This water, as steam, transports the target analyte from the solid to the nonabsorbing solvent. In this type of work, the sample is a good dielectric while the solvent is a poor dielectric. The microwave process gave higher yields than the traditional steam distillation process. The authors postulated that the improved efficiency was due to the lower bulk temperatures and shorter extraction times.

In 1992, Bichi *et al.* published work from the pharmaceutical area based on MAE using closed-vessel technology with temperature-feedback control. They extracted pyrrolizidine alkaloids from dried plants using 25–50 mL of methanol. The extractions were performed over a temperature range of 65–100°C for 20–30 min. The MAE technique gave qualitatively and quantitatively identical chromatographic results relative to the samples extracted by the Soxhlet procedure with a significant reduction in extraction time and solvent consumption. The temperature feedback control provided highly reproducible extractions.

In 1993, Onuska and Terry published the first data on the use of MAE for pollutants from environmental samples. They successfully extracted organochlorine pesticide residues from soils and sediments using a 1 : 1 mixture of isooctane–acetonitrile using sealed vials. The samples were irradiated for five 30 s intervals. This produced faster and more reliable results than the conventional methods. They also studied the use of a nonpolar solvent, iso-octane, for the extraction of wet sediment samples. Pesticide residue recoveries increased as the moisture content increased to a maximum of 15% and then levelled off. This shows the importance of a polar co-solvent when using a nonpolar extracting solvent for MAE techniques that are temperature-dependent. In 1994 Lopez-Avila *et al.* published their work to expand the use of MAE to 187 volatile and semivolatile organic compounds from soils. The compounds included polyaromatic compounds (PNA), phenols, organochlorine pesticides and organophosphorus pesticides. This work was performed using a temperature feedback-controlled microwave heating system with closed vessels. This demonstrated the viability of the technique for the extraction of many compounds of interest to the US Environmental Protection Agency with relatively small volumes of solvent and extraction times of only 10 min. This culminated in 1998 with the approval of the microwave extraction technique by the SW-846 Organic Methods Workgroup of incorporation into SW-846 as the latest of the 'green' methods.

In 1997 there were a number of significant extensions of the technology. Incorvia-Mattina *et al.* investigated the use of MAE to extract taxanes, used in ovarian and breast cancer research, from Taxus biomass. MAE offered significant time savings versus the standard shaking technique and improved yields versus synthetic approaches. Stout *et al.* combined MAE with liquid chromatography–electrospray ionization–mass spectrometry for the determination of imidazolinones in soils at concentrations of less than 1 p.p.b. McNair *et al.* reported the combination of MAE with solid-phase microextraction for the analysis of flavour ingredients at concentrations of 2–10 p.p.b. in solid food samples. This technique showed good selectivity for the target analytes in a variety of foods.

Instrumentation

MAE is the process of heating solid sample–solvent mixtures with microwave energy and the subsequent partitioning of the compounds of interest from the sample to the solvent. The most common approach is to perform the extraction in a sealed vessel that is microwave-transparent. This allows a temperature elevation significantly above the atmospheric boiling point of the solvent (**Table 1**) and hastens the extraction process.

The alternative approach is to perform the extraction in an open vessel at atmospheric conditions. This approach is common when the solvent is nonpolar or microwave-transparent and the sample is a biological or agriculture tissue that has a polar constituent, usually water. This is the basis for the patent issued to Environment Canada for the MAP^{TM}.

For the scope of this review article, we will limit the instrumentation discussion to the closed-vessel microwave heating system. In approaching MAE

Table 1 Solvent boiling point – closed vessel temperature comparison

Solvent	*Boiling point (°C)*	*Closed vessel temperature (°C)*[a]
Acetone	56.2[b]	164
Acetone : Cyclohexane 7 : 3 vol/vol	52	160
Acetonitrile	81.6[b]	194
Dichloromethane	39.8[b]	140
Hexane	64.7[b]	162[c]
Methanol	68.7[b]	151

[a] At 175 psig.

[b] *Lange's Handbook of Chemistry*, 14 edn. Dean JA (ed.) New York: McGraw-Hill, Inc., 1992: 11.10–11.12.

[c] Using carboflon heating insert.

applications, it is first necessary to understand the analysts' objectives. These objectives include:

1. Selection of the optimum solvent for the analytes of interest.
2. Minimization of any steps prior to the extraction step.
3. The use of a minimum amount of solvent for the extraction step.
4. Effective and reproducible extraction conditions.
5. High sample throughput.
6. Safe operation.

Considering these objectives, a microwave-assisted extraction system was designed that was a derivative of the successful microwave acid digestion system. The key components are:

1. Microwave instrumentation
2. Solvent safety features
3. Vessel technology
4. Temperature control system
5. Indirect heating source (for heating nonpolar solvents)
6. Stirring mechanism

Each component is reviewed in terms of its technical merit and how it assists in meeting the analysts' objectives for the extraction step.

Microwave Instrumentation

Figure 1 shows the major components of the microwave system, including the magnetron, isolator, wave guide, cavity and mode stirrer. Microwave energy is generated by the magnetron, propagated down the wave guide and introduced into the cavity. The mode stirrer distributes the energy in various directions while the cavity acts as a containment housing for the energy until it is absorbed by the sample load within the cavity. The isolator protects the magnetron from

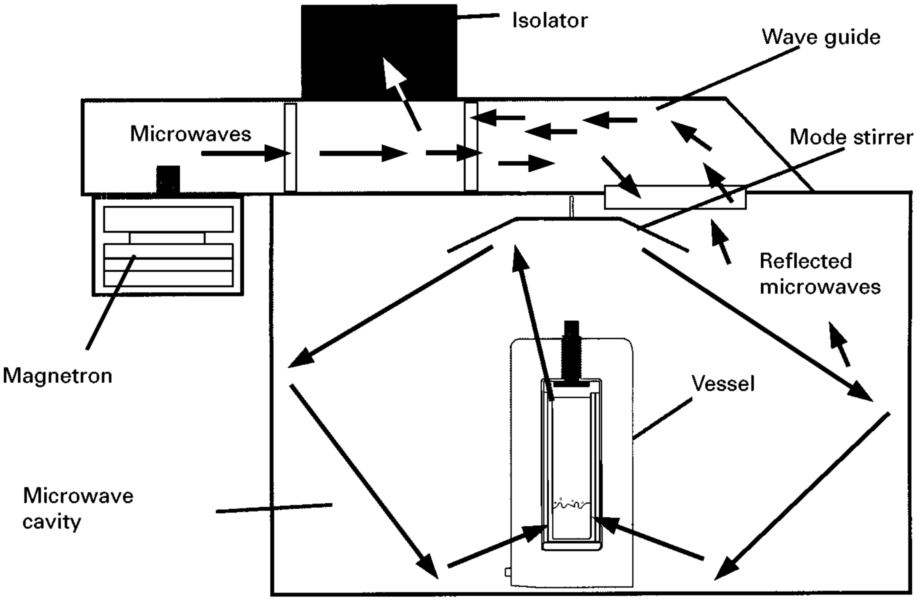

Figure 1 Microwave system components.

reflected energy that would decrease its power output. A good analogy is a one-way mirror – it allows energy to go from the magnetron to the cavity but will not allow it to go from the cavity to the magnetron. A turntable can be used to rotate the sample load within the cavity to ensure even energy distribution.

Microwave heating is significantly different from conductive heating methods. Conductive heating is sample-independent. All samples placed inside a conduction heating oven will equilibrate to the programmed temperature. This can take quite some time. Microwave heating is sample-dependent. The temperature rise rate of samples will depend on their microwave-absorbing characteristics. The microwave design provides the ability to heat uniformly a large number of samples in a short period of time based on the sample load characteristics.

Solvent Safety Features

Due to the flammable characteristics of many organic solvents, there is a major safety issue when heating a solvent in a microwave field. This safety issue is magnified when heating these solvents in sealed vessels at temperatures up to 100°C above their atmospheric boiling point. The microwave system should have redundant safety features, each acting as a back-up to prevent possible fire or explosion from occurring inside the cavity. Instruments should be designed to eliminate possible ignition sources, to detect solvent leaks and to remove leaking solvent. **Figure 2** is an illustration of the interior of a commercially available microwave extraction system. The safety aspects are an exhaust fan which evacuates the cavity air volume approximately once per second. If the exhaust fan fails or there is a block downstream of the fan, the air flow switch shuts down the system. The solvent detector monitors the cavity for the presence of solvent. The detector shuts the system down if solvent concentrations reach one-tenth of the lower explosive limit for acetone, sets off an alarm and posts a message for the operator. The cavity is Teflon-coated to minimize the potential of high energy discharges. The system door is designed to withstand an event equivalent to the explosion of 1 g of TNT. It will partially open, allowing gases to escape, and then the compression springs will pull it closed. This will contain any of the contents associated with a vessel-related event inside the system's cavity.

Vessel Technology

The closed vessels used for MAE are designed for temperatures up to 200°C and pressures of 200 psi (14 bar). The materials of construction for the components that are in contact with the sample–solvent mixture, either Teflon or glass, are inert to solvents. However, since these materials are relatively weak, an outer body is used that is much stronger – either a reinforced thermoplastic or a frame of polypropylene, or both. In addition, these materials of construction absorb a minimal amount of microwave energy. **Figure 3** illustrates a standard extraction vessel and a control extraction vessel. The vessels are composed of glass or Teflon liners, Teflon, PFA® seal cover, polyetherimide load disc and sealing screw, glass-filled polyetherimide sleeve and polypropylene support frame. The vessel has a built-in pressure relief

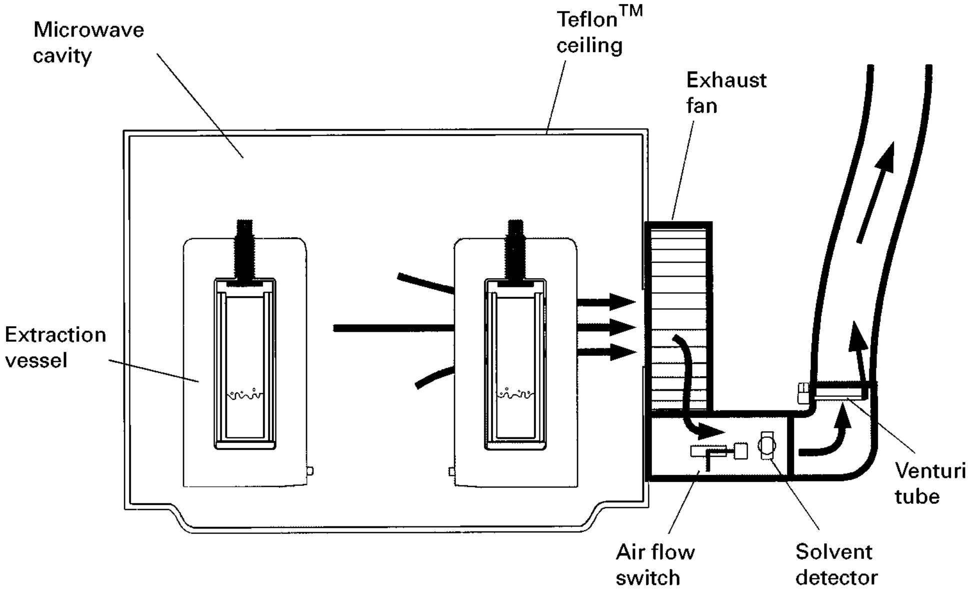

Figure 2 Safety exhaust and solvent detector.

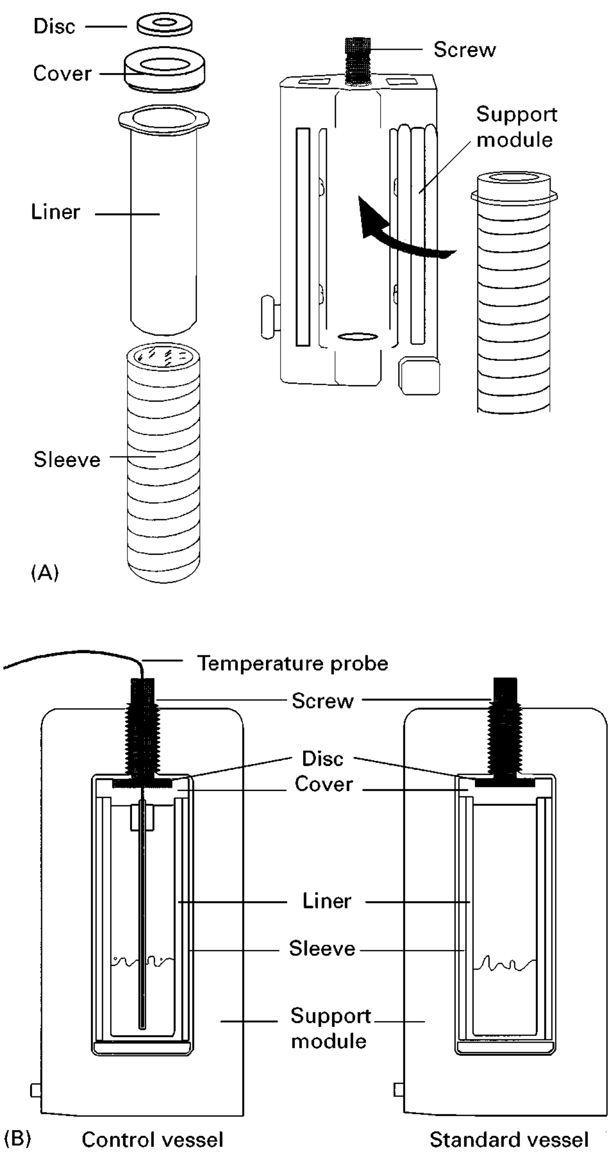

Figure 3 (A) Exploded view of a microwave extraction vessel. (B) Cut-away view of microwave extraction vessels.

mechanism for safety purposes. If the pressure inside the vessel exceeds the operating limits, the vessel will automatically vent. The control vessel is modified to accept a probe to monitor and control the extraction temperature. A turntable of 14 extraction vessels is placed into the instrument's cavity for batch processing. The ability to rapidly achieve elevated solvent temperatures under controlled conditions for a large batch of samples is a major advantage of the MAE technique.

Temperature Control System

Temperature control is necessary to optimize the extraction efficiency, prevent thermal degradation of the target analytes, and to provide reproducible operating conditions. This is achieved with a temperature measurement system that is microwave-transparent so it does not cause any self-heating. The temperature probe is inserted directly into the control vessel to measure the temperature of the solvent–sample mixture. It is then used in a feedback control loop to regulate the microwave power output to achieve and maintain the operator-selected extraction temperature. This approach provides temperature control for one of the samples in the batch and assumes equivalent reaction conditions for all the other samples. This control technique is augmented with an indirect infrared temperature measurement system. The infrared sensor is located underneath the cavity floor. It monitors the temperature of each vessel as it passes over the sensor. This temperature reading is correlated to the direct temperature reading from the control vessel to provide temperature data for all of the samples in the batch.

Indirect Heating Source

In some extraction applications, the solvent of choice for the target analytes is nonpolar and therefore does not heat when exposed to the microwave field. This would normally preclude the use of the MAE technique unless the analyst is willing to alter the solvent scheme to include a polar co-solvent. This is not desirable since it alters the extraction efficiency (or selectivity). This problem is overcome with the use of an insert that is a chemically inert fluoropolymer filled with carbon black, a strong microwave absorber. The insert is placed into the vessel with the solvent–sample mixture. The insert absorbs microwave energy and transfers the thermal energy it generates to the mixture. The performance characteristics of the heating inserts for heating with *n*-hexane in a microwave field are seen in **Table 2**. The use of the heating insert allows transfer of existing methods to the MAE technique without a change in the solvent scheme.

Stirring Mechanism

Stirring increases the surface area contact between the sample and solvent. This offers the benefit of improved extraction efficiency and decreased solvent consumption. Stirring is achieved with the use of a rotating magnet below the cavity floor and the placement of a magnetic spinbar in the extraction vessel. The magnet creates a rotating magnetic field that couples with the spinbar in the vessel to create a stirring effect. The spinbars are either coated with an unfilled fluoropolymer for applications using polar solvents or a carbon black filled fluoropolymer for applications with nonpolar solvents.

Table 2 MAE heating rate for *n*-hexane using heating insert

Temperature set point (°C)	*Time to temperature (min)*		
	1 Vessel	*6 Vessels*	*12 Vessels*
100	1:45	2:45	3:45
125	2:30	4:15	6:15
150	3:35	6:15	9:30

Notes: Each vessel contained 50 mL of *n*-hexane and 1 heating insert. The starting temperature was 25°C. As a point of reference, 12 vessels containing 50 mL of acetone will reach 125°C in 5 min under equivalent conditions.

These components create a system to perform MAE. This technique has the capability to reduce extraction time, reduce solvent consumption and improve extraction efficiency. However, it does not come without a cost. The extraction step is but one of many necessary steps to obtain the final analytical result. The analyst must take into account the differences in using the MAE technique versus their existing approach. The primary difference is the finished sample form. It is the same sample–solvent mixture originally placed into the vessel. The analyst needs to separate the sample from the solvent at the completion of the extraction step. If the analyst can work with an aliquot of the solvent for analysis, this objection can be overcome with the use of a syringe filtration technique. A secondary consideration is the vessel manipulations used with MAE. These manipulations will be new for the analyst.

Applications

MAE has been applied to a wide variety of samples in which traditional Soxhlet extractions are performed. **Table 3** gives a performance comparison of MAE versus conventional extraction techniques for a variety of sample types. For the scope of this work, we will limit the application discussion to the extraction of plastics and polymers, pesticides and environmental samples.

Plastics and Polymers

The additive package used in the production of polyolefins is designed to improve processing efficiency or to impart specific performance characteristics. The package can contain antioxidants, antistatic agents, slip agents, anti-block agents, UV stabilizers or antifogging agents. It is important for production efficiency and product quality that the appropriate amount of each additive is present. A fast and reliable method is needed to determine the additive concentration level. The conventional extraction approach is a reflux technique with an appropriate solvent for 1–48 h followed by high performance liquid chromatography (HPLC) analysis. An alternative extraction technique is sonication for 30–60 min, but the gain in speed is offset by a loss in extraction efficiency. MAE has the ability to address the time and extraction deficiencies of the reflux and sonication techniques. Freitag and John first demonstrated the potential for MAE when they obtained excellent antioxidant recoveries from polypropylene and polyethylene.

Pesticides and Herbicides

Pesticides and herbicides are used to protect a wide variety of agricultural commodities. There is an interest in pesticides, herbicides and their degradation product concentrations in plant and animal tissues and soil and sediment samples. The underlying assumption is that extractable compounds are labile in the environment and constitute a threat to the environment if they are hazardous. Specific examples are from the work of Fish and Revesz on chlorinated pesticides from soils and the work of Stout's group on imidazolinone herbicides in plant tissues. Fish and Revesz showed chlorinated pesticide recoveries from a Certified Reference Soil greater than or equal to those achieved using the standard Environmental Protection Agency Soxhlet technique, method 3540. This was achieved with extraction times of only 20 min and solvent volumes of 50 mL. Stout *et al.* incorporated the use of MAE with liquid chromatography–electrospray ionization–mass spectrometry to shorten the clean-up procedure and method development time of residue methodologies for determining the imidazolinones and their metabolites in crops. This application area is of concern not only to the traditional commercial testing laboratory, but to agro-chemical producers.

Environmental

The organic side of the environmental laboratory market constitutes the majority of the analytical testing load. The extraction of priority pollutants, as well

Table 3 MAE versus conventional extraction techniques

Sample	*Analyte*	*Microwave*			*Conventional*		
		Solvent volume (mL)	*Extraction time (min per sample)*[a]	*Concentration*	*Solvent volume (mL)*	*Extraction time (min per sample)*[a]	*Concentration*
Environmental							
Method 3546	Priority pollutants	25	7	–	300	1080	–
Soil	TPH	30	7	943 mg kg^{-1}	300	60	773 mg kg^{-1}
Soil	OCP	50	7	92.3%[b]	300	1080	83.4%[b]
Soil	PCBs	25	7	47.7 μg g^{-1}	250	1080	44.0 μg g^{-1}
Sediment	Methylmercury	10	6	80 μg g^{-1}	200	150	81 μg g^{-1}
Sediment	Dioxins	30	8	565 pg g^{-1}	300	1440	542 pg g^{-1}
Biomaterial	Organotin	20	5	1.28 μg g^{-1}	–		1.3 μg g^{-1}
Plastics							
HDPE	Anti-oxidants	30	6	157 μg g^{-1}	200	60	140 μg g^{-1}
LDPE	Erucamide	30	6	480 μg g^{-1}	200	60	491 μg g^{-1}
PET	Oligomers	40	8.5	1.16%	190	1440	1.24%
Nylon	Plasticizer	30	8	11.80%	200	120	11.78%
Polyamide	% Extractables	35	8	6.62%	150	960	6.60%
Cellulose acetate	% Oil	50	6	2.42%	135	250	2.40%
Agrochemical							
Radish	PCNB	25	6	0.42 p.p.b.	300	60	0.36 p.p.b.
Soil	Imidazolinone	20	5	11.2 p.p.b.	400	120	10 p.p.b.
Food							
Corn	% Fat	40	7	49.02%	75	360	49.11%
Feed	% Fat	35	6	8.73%	250	120	8.75%
Other							
Fibre glass	% Extractables	75	7	43.80%	200	120	39.00%
Paper	% Wax	50	5	0.88%	150	60	0.71%
Carbon fibre	% Extractables	60	7	0.55	250	1080	0.55%

[a] Includes weighing, reagent addition, vessel manipulation, heating and cooling time.

[b] Value is % recovery. TPH, total petroleum hydrocarbons; OCP, organochlorine pesticides; PCB, polychlorinated biphenyls; HDPE, high density polyethylene; LDPE, low density polyethylene; PET, polyethylene terephthalate; PCNB, pentachloronitrobenzene.

as other organic molecular species, from solid samples is a primary concern. The workload in the environmental laboratory is expected to increase significantly and will thus require extraction techniques that offer increased throughput, reduced solvent consumption, improved efficiency and reproducibility. MAE has the potential to address these needs. McMillin of US Environmental Protection Agency-Region VI demonstrated this in a comparison of various soil extraction techniques for semivolatile analysis. He used an abbreviated MAE technique consisting of a small modification to regular MAE. He worked with only 10 mL of solvent versus the conventional 30 mL. This eliminated the subsequent concentration step and allowed the sample to be injected straight from the extraction vessel into a GC. The abbreviated MAE provided better extraction efficiencies and reproducibility than the three conventional techniques. The extraction time averaged 16 min per sample with a solvent use of 10 mL per sample.

One difficulty in the use of MAE for the environmental laboratory market is Environmental Protection Agency approval. The methodology has been approved by the SW-846 Organic Methods Workgroup for incorporation into SW-846. However, the method has not been promulgated and thus can only be used when regulations do not specifically require SW-846 methods.

Future Developments

The instrumentation for MAE will continue to evolve, as will its potential applications. There will be developments in the vessel technology to address the separation issue of the sample and solvent after the extraction step. This will allow the technique to be a true replacement for the Soxhlet. There is also a need for larger vessel sizes. The current vessel has a working volume of 100 mL. There is a need to increase this to 250 mL and even higher for bulky samples and the inevitable push for lower detection limits. Finally, the microwave system's use should be extended to concentration of the sample after the extraction step. This will create a multi-tasking tool for the analytical laboratory.

MAE has focused on extraction applications from solid matrices. However, its speed and efficiency suggest that this technique will be used for isolating pharmaceutical compounds during the drug discovery process. The recent addition of sample stirring suggests that it can be extended to liquid–liquid extraction applications. It could also be coupled with solid-phase microextraction to lower detection limits significantly. As MAE becomes more widely accepted and instrumentation evolves, we should see a significant increase in its applicability.

See also: **II/Extraction:** Microwave-Assisted Extraction. **Environmental Applications:** Soxhlet Extraction.

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