Recent Extraction Techniques for Natural Products: Microwave-assisted Extraction and Pressurised Solvent Extraction

Beatrice Kaufmann and Philippe Christen*
University of Geneva, School of Pharmacy, Laboratory of Pharmaceutical Analytical Chemistry, 20 bd d’Yvoy, CH-1211 Geneva 4, Switzerland

In the last 10 years there has been an increased interest in using techniques involving microwave-assisted extraction and pressurised solvent extraction in analytical laboratories. This review gives a brief overview of both methods, and reports on their application to the extraction of natural products. The influence of parameters such as the nature of the solvent and volume, temperature, time and particle size of the matrix is discussed. Through numerous examples, it is demonstrated that both techniques allow reduced solvent consumption and shorter extraction times, while the extraction yields of the analytes are equivalent to or even higher than those obtained with conventional methods. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: Microwave-assisted extraction; solid–liquid extraction; supercritical fluid extraction; pressurised solvent extraction; accelerated solvent extraction; enhanced solvent extraction; natural products.

INTRODUCTION

A broad spectrum of solid–liquid extraction (SLE) techniques is widely used for the early purification of natural products from plant material and micro-organisms. Classically, SLE can be divided into traditional and recent methods. Traditional methods include Soxhlet extraction, maceration, percolation, turbo-extraction (high speed mixing) and sonication. These techniques have been used for many decades; however, they are very often time-consuming and require relatively large quantities of polluting solvents. Supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE) are fast and efficient unconventional extraction methods developed for extracting analytes from solid matrices. Numerous review articles and textbooks have been published on SFE (Lee and Markides, 1990; Westwood, 1993; King and Bott, 1993) and a number are more particularly dedicated to SFE of natural products (Castioni et al., 1995; Smith, 1995, 1996; Jarvis and Morgan, 1997). The present review deals with the application of MAE and PSE to the extraction of secondary metabolites from plant material.

MICROWAVE-ASSISTED EXTRACTION

Microwaves have been used since World War II following the development of radar technology, and later the first commercial application of microwaves concerned domestic ovens. The use of microwave energy as a heating source in analytical laboratories started in the late 1970s and was applied to acid digestions (Abu Samra et al., 1975). The development of microwave assisted extractions was first reported by Ganzler and co-workers (Ganzler et al., 1986a; Ganzler and Salgó, 1987).

Principle of the method and heating mechanism

Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz (Camel, 2001). In order to avoid interferences with radio communications, domestic and industrial microwaves generally operate at 2.45 GHz (Fig. 1). Owing to their electromagnetic nature, microwaves possess electric and magnetic fields which are perpendicular to each other. The electric field causes heating via two simultaneous mechanisms, namely, dipolar rotation and ionic conduction (Thuéry, 1992; Demesmay and Olle, 1993; Sinquin et al., 1993). Dipolar rotation is due to the alignment on the electric field of the molecules possessing a dipole moment (either permanent or induced by the electric field) in both the solvent and the solid sample. This oscillation produces collisions with surrounding molecules and thus the liberation of thermal energy into the medium. With a frequency of 2.45 GHz, this phenomenon occurs 4.9 × 109 times per second (Ganzler et al., 1990; Sinquin et al., 1993; Barnabas et al., 1995; Onuska and Terry, 1995) and the resulting heating is very fast. Indeed, the larger the dielectric constant of the solvent (see Table 1), the more optimal the heating (Jassie et al., 1997). Consequently, unlike classical conductive heating methods, microwaves heat the whole sample simultaneously (Fig. 2). In the case of extraction, the advantage of microwave heating is the disruption of weak hydrogen bounds promoted by the dipole rotation of the molecules. A higher viscosity of the medium lowers this mechanism by affecting molecular rotation (Sinquin et al., 1993; Camel and Bermond, 1999). Furthermore, the migration of dissolved ions increases solvent penetration into the
matrix and thus facilitates the solvation of the analyte (Ganzler et al., 1990). Ionic currents are also induced in the solution by the electric field. As the medium resists these currents, frictions occur and heat is liberated by a Joule effect. This phenomenon depends on the size and charge of the ions present in the solution.

The effect of microwave energy is strongly dependent on the nature of both the solvent and the solid matrix. Solvents generally used cover a wide range of polarities, from heptane to water. Most of the time, the chosen solvent possesses a high dielectric constant and strongly absorbs microwave energy, however, the extracting selectivity and the ability of the medium to interact with microwaves can be modulated by using mixtures of solvents (Renoe, 1994). In some cases, the matrix itself interacts with microwaves while the surrounding solvent possesses a low dielectric constant and thus remains cold (Jassie et al., 1997). This latter situation presents some obvious advantages in the case of thermosensitive compounds and has been successfully used for the extraction of essential oils (Paré, 1990, 1991; Chen and Spiro, 1994; Romele and Polesello, 1997). Indeed, microwaves interact selectively with the polar molecules present in glands, trichomes or vascular tissues. Localised heating leads to the expansion and rupture of cell walls and is followed by the liberation of essential oils present in glands, trichomes or vascular tissues. Localised heating leads to the expansion and rupture of cell walls and is followed by the liberation of essential oils present in glands, trichomes or vascular tissues.

Table 1. Dielectric constants and dipole moment values of some commonly used solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Dielectric constant (20°C)</th>
<th>Dipole moment (25°C) (Debye)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>1.89</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.4</td>
<td>0.36</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>8.9</td>
<td>1.14</td>
</tr>
<tr>
<td>Acetone</td>
<td>20.7</td>
<td>2.69</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24.3</td>
<td>1.69</td>
</tr>
<tr>
<td>Methanol</td>
<td>32.6</td>
<td>2.87</td>
</tr>
<tr>
<td>Water</td>
<td>78.5</td>
<td>1.87</td>
</tr>
</tbody>
</table>

control of the water content of the matrix allows more reproducible results.

Instrumentation

Two types of instruments are commercially available and they use different approaches. The most common procedure involves extraction in a closed vessel under controlled pressure and temperature, whilst an alternative approach uses an open extracting vessel under atmospheric pressure. It must be strongly stressed that using a domestic microwave oven for laboratory purposes should not be considered. Application of microwave energy to highly flammable organic solvents may cause serious hazards. Furthermore, reproducibility may be poor with a domestic device because of the lack of homogeneity of the microwave field. It is therefore seriously recommended that only equipment approved for MAE be used.

Closed vessel systems. Such systems are generally advised for digestions or acid mineralisations or for extractions under drastic conditions, since the solvents may be heated to ca. 100°C above their atmospheric boiling point (Barnabas et al., 1995; Jassie et al., 1997). Both extraction speed and efficiency are enhanced in this procedure. Hazards occasioned by heating highly flammable solvents are overcome through the use of recent security techniques such as high capacity exhaust fans, solvent vapour detectors, or pressure-burst safety membranes placed on each vessel (Demesmay and Olle, 1993; Jassie et al., 1997). Figure 3 shows a schematic diagram of a closed vessel system from CEM Corporation (Matthews, NC, USA). In order to overcome the non-homogeneity of the field, the cells are placed on a rotating carousel as in a domestic oven. The solvents can be varied, and the pressure in the vessels essentially depends on the volume and boiling point of the solvents used. The temperature can be set at a fixed value by adjusting the microwave power. Typically, the cells are made of Teflon. In closed vessel systems, the maximal power delivered is about 600–1000 W (Paré, 1990, 1991; Young, 1995), but the chosen power has to be set correctly to avoid excessive temperatures leading to possible solute degradation and overpressure problems. The vessel must be cooled to room temperature before opening: this is particularly important in the presence of volatile solutes which can partition into the head-space, but this step can considerably increase the overall...
 extraction time. Furthermore, an additional filtration or centrifugation step is necessary in order to remove the solid residue.

**Open cells.** These cells are quartz vessels topped by a solid residue. A centrifugation step is necessary in order to remove the extraction time. Furthermore, an additional filtration or centrifugation step is necessary in order to remove the solid residue.

**Closed vessels**

Pyrimidine glycosides. The first papers to report the use of MAE for natural products were published by Ganzler and co-workers (Ganzler et al., 1986a, b; Ganzler and Salgò, 1987) and concerned the extraction of vicine and convicine from faba beans: these toxic pyrimidine glycosides preclude the use of faba beans as a source of nutritional proteins. Ground beans were suspended in a methanol:water mixture (1:1, v/v), and the suspension was subjected to two successive microwave irradiations (30 s each) with a cooling step in between. No degradation could be observed under these conditions, but further irradiation was found to decrease the yield of vicine and convicine. The yield obtained was 20% higher than with the conventional Soxhlet extraction method.

**Gossypol.** As for faba beans, the presence of gossypol in cotton seeds limits their use for human consumption or animal feeding (Ganzler et al., 1986a; Ganzler and Salgò, 1987). Extraction yields of gossypol were much higher with three cycles of 30 s each of MAE than with the traditional 4 h Soxhlet extraction, probably because the compound is sensitive to high temperatures. This phenomenon was confirmed by further MAE irradiations which also lead to the degradation of gossypol. Alkaloids. Sparteine, a lupine alkaloid, was extracted from lupine seeds (Lupinus mutabilis) with methanol:acetic acid (99:1, v/v) in a domestic microwave oven (see caution above!), and the treatment (one to five cycles of 30 s with a cooling step in between) gave 20% more sparteine than was obtained with a shaken-flask extraction using the same solvent mixture for 20 min (Ganzler et al., 1990). Pyrrolizidine alkaloids were extracted from dried plant material (Senecio sp.) by Bicchi et al. (1992) using closed vessel technology with temperature feed-back control (65–100°C). Qualitative and quantitative chromatographic results were identical to those obtained with traditional techniques, with a significant reduction in extraction time and solvent consumption with good reproducibility.

**Terpenes.** Five terpenic compounds (linalool, α-terpineol, citronellol, nerol and geraniol) associated with grape (Vitis vinifera) aroma were extracted from must samples by MAE (Carro et al., 1997). Four variables (extracting solvent volume, extraction temperature, amount of sample and extraction time) were optimised by means of two- and three-level factorial designs. Several conditions were fixed, such as the extraction time (10 min) and the applied power (475 W). The solvent volume appeared to be the only statistically significant factor, but was limited to 15 mL by the cell size. The highest extraction yield was obtained with both the solvent volume and the temperature at their maximum tested values. In contrast, the sample amount had to be minimised in order to obtain the best recoveries. The final

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**Figure 3.** Schematic diagram of a closed-vessel microwave system for extraction.

**Figure 4.** Schematic diagram of an open focused-microwave system for extraction.
Table 2. Application of MAE to natural product extraction

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Matrix</th>
<th>System</th>
<th>Extraction conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vicine, convicine</td>
<td>Faba beans (Vicia faba)</td>
<td>Domestic oven</td>
<td>Methanol:water (1:1); two successive irradiations (30 s) with an intermediate cooling step</td>
<td>Ganzler et al. (1986a, 1986b); Ganzler and Salgò (1987)</td>
</tr>
<tr>
<td>(pyrimidine glycosides)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gossypol</td>
<td>Cotton seeds</td>
<td>Domestic oven</td>
<td>Three cycles of irradiation (30 s) with cooling steps in between</td>
<td>Ganzler et al. (1986a); Ganzler and Salgò (1987)</td>
</tr>
<tr>
<td>Spartheine (alkaloid)</td>
<td>Lupine seeds</td>
<td>Domestic oven</td>
<td>Four cycles (30 s) with cooling steps in between</td>
<td>Ganzler et al. (1986b, 1990)</td>
</tr>
<tr>
<td>Terpenes (linalool, terpineol, terpinol, nerol and geranial)</td>
<td>Must (Vitis vinifera)</td>
<td>Closed vessels</td>
<td>10 mL dichloromethane; 475 W; 10 min; 90°C Hexane</td>
<td>Carro et al. (1997)</td>
</tr>
<tr>
<td>Essential oils</td>
<td>Mentha piperita, Thuya occidentalis</td>
<td>Modified domestic</td>
<td>Essential oils: Hexane, carbon tetrachloride, toluene; 750 W; &lt;60 s</td>
<td>Paré (1994)</td>
</tr>
<tr>
<td>Essential oils</td>
<td>Rosemary and peppermint leaves</td>
<td>Domestic oven</td>
<td>Essential oils: “High cooking level”; 5 min</td>
<td>Chen and Spiro (1994)</td>
</tr>
<tr>
<td>Essential oils</td>
<td>Plant leaves</td>
<td>Domestic oven</td>
<td>Essential oils: “High cooking level”; 5 min</td>
<td>Collin et al. (1991)</td>
</tr>
<tr>
<td>Essential oils</td>
<td>Fresh leaves of Lippia siodiens</td>
<td>Domestic oven</td>
<td>Essential oils: Hexane; &lt;60 s</td>
<td>Craveiro et al. (1989)</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Paprika powder</td>
<td>Closed vessels</td>
<td>Carotenoids: 50 W; 120 s; &lt;60°C</td>
<td>Csikunadi Kiss et al. (2000)</td>
</tr>
<tr>
<td>Taxanes (paclitaxel)</td>
<td>Needles of Taxus sp.</td>
<td>Closed vessels</td>
<td>Taxanes (paclitaxel): 5 g fresh needles pre-soaked with 5 mL water prior to extraction with 10 mL of 95% ethanol; 100% power; 54 s; 85°C</td>
<td>Incovia Mattina et al. (1997)</td>
</tr>
<tr>
<td>Ergosterol Withanolides</td>
<td>Fungal contaminations</td>
<td>Domestic oven</td>
<td>Ergosterol Withanolides: 375 W; 35 s 100 mg material pre-soaked with 0.6 mL water prior to extraction with 5 mL methanol; 25 W; 40 s</td>
<td>Young (1995)</td>
</tr>
<tr>
<td>Cocaine and benzoylecgonine Alkaloids</td>
<td>Erythroxylum coca leaves</td>
<td>Open cell, focused</td>
<td>Cocaine and benzoylecgonine Alkaloids: Methanol; 125 W; 30 s</td>
<td>Brachet et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>Senecio sp.</td>
<td>Closed vessels</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Optimised extraction conditions were as follows: 5 mL sample amounts extracted with 10 mL of dichloromethane at a temperature of 90°C for 10 min with the microwave power set at 50% (475 W).

**Essential oils.** MAE of essential oils has been patented by Paré (1991). The samples were suspended in hexane and the microwaves reached the inner glandular and vascular systems of the plant material. Owing to the high moisture content of these structures they were heated almost specifically and this promoted disruption of cell membranes releasing the analytes into the solvent. The same author also patented the extraction of volatiles from biological material (Paré, 1990); the method involved the exposure of oil-containing cellular matter or glandular systems to microwaves and the dissolution of essential oil in a suitable organic solvent.

Another patent has been deposited by Paré (1994) concerning the extraction of volatile oils from plants and biological material. This procedure was applied by Chen and Spiro (1994) for the extraction of essential oils from rosemary and peppermint leaves suspended in hexane, ethanol or mixtures of the two solvents. Scanning electron micrographs of the leaves showed structural changes in the oil-containing glands after microwave extraction: some of them were found to be collapsed and others completely disintegrated. Two distinct extraction mechanisms are plausible, one involving diffusion of the essential oil across the gland wall and the other involving rupture of the gland and liberation of the constituents into the solvent. Either of the two phenomena may be prominent according to the different maturation stages of the glands.

MAE has also been compared to classical hydrodistillation for the extraction of essential oils from 10 different plant species using a domestic microwave oven (Collin et al., 1991). The yields were generally similar, but the chromatographic profiles varied dramatically, especially with respect to the ratios between the different substances.

The microwave technique was used to promote desorption of essential oil from Lippia siodiens with a modified commercial microwave oven (Craveiro et al., 1989). Fresh leaves were irradiated in the oven and the
volatiles were extracted by an air current passing through the system. The yields obtained after 5 min were comparable to those obtained after 60–90 min of steam distillation, and both qualitative and quantitative performances were found similar to the conventional method.

**Carotenoids.** The MAE of carotenoids from paprika has been optimised by Csiktusnadi Kiss et al. (2000). Thirty different water:organic solvent mixtures were evaluated, and both extraction efficiency and selectivity were significantly dependent on the dielectric constant of the extracting solvent mixture. The extractions had to be performed at temperatures lower than 60°C because of the possible rearrangement of molecules at higher temperatures.

**Steroids.** Recently, a system has been developed simultaneously to saponify and extract ergosterol by MAE (Young, 1995). The determination of this compound in filtered air or contaminated corn or dust can be used as an indicator of fungal contamination. The samples were placed in culture tubes containing 2 mL methanol and 0.5 mL 2 M sodium hydroxide. Microwave irradiation was applied at 375 W for 35 s and the samples were cooled for 15 min before neutralisation with 1 M hydrochloric acid followed by pentane extraction. It was demonstrated that only 30–40 s were sufficient to extract ergosterol quantitatively and that the yield was similar to or even higher than that obtained with the traditional methanolic extraction followed by alkaline saponification and pentane extraction.

**Taxanes.** The application of microwave energy to the extraction of taxanes from Taxus biomass was reported by Incorvia Mattina et al. (1997). Various parameters, including temperature, extraction time, solvent choice and water content were investigated in order to optimise extraction efficiency. Recoveries of taxane reached 100% of the conventional method when the biomass was freeze-dried to less than 10% moisture and pre-soaked with water prior to extraction using 95% ethanol. As some degradation of paclitaxel occurred at temperatures above 115°C, the temperature was set at 85°C and MAE was performed for 54 s. The extracts were quantitatively and qualitatively equivalent to those obtained with the conventional extraction method, but with considerable reduction of both extraction time and solvent consumption.

**Open vessels**

Very few papers have been published concerning the focused microwave extraction of natural products.

**Alkaloids.** Cocaine and benzoylecgonin were extracted from coca leaves (100 mg) by focused MAE (Brachet et al., 2002). The extraction was optimised by taking into account several parameters such as the nature of the extracting solvent, particle size distribution, sample moisture, applied microwave power and radiation time. MAE was found to generate similar extracts to those obtained by conventional SLE but in a more efficient manner. Indeed, 30 s were sufficient to extract cocaine quantitatively from leaves, using methanol as solvent and a microwave power of 125 W.

**Steroids.** Focused MAE was applied to the extraction of withaferin A, iochromolide and withacnatin from the leaves of Iochroma gesnerioides (Kaufmann et al., 2001a). Withanolides are steroidal lactones derived from an ergostane-type skeleton. Six extraction variables, i.e. the nature and volume of the extracting solvent, sample moisture, extraction time, power of irradiation and particle size of the matrix were investigated. The most favourable conditions were obtained using powdered plant material (<220 μm), previously moistened with water for 15 min, and extracted with methanol for 40 s at 25 W. The extraction yield obtained with the optimised method was comparable to that achieved with a Soxhlet extraction.

### PRESSURISED SOLVENT EXTRACTION

Pressurised solvent extraction (PSE) is a SLE technique which has been developed as an alternative to current extraction methods such as Soxhlet, maceration, percolation or reflux, offering advantages with respect to extraction time, solvent consumption, extraction yields and reproducibility. PSE uses organic solvents at elevated pressure and temperature in order to increase the efficiency of the extraction process. Increased temperature accelerates the extraction kinetics and elevated pressure keeps the solvent in the liquid state, thus enabling safe and rapid extractions. Furthermore, high pressure forces the solvent into the matrix pores and hence should facilitate extraction of analytes. High temperatures decrease the viscosity of the liquid solvent, allowing a better penetration of the matrix and weakened solute–matrix interactions. In addition, elevated temperatures enhance diffusivity of the solvent resulting in increased extraction speed.

#### Instrumentation

ASE® (accelerated solvent extraction) is a form of PSE and the first instrument was commercialised by the Dionex Corporation (Sunnyvale, CA, USA) in 1994 (Richter et al., 1996; Reighard and Olesik, 1996). More recently, ESE (enhanced solvent extraction) or PSE instruments have appeared (Bautz et al., 1998).

**Accelerated solvent extraction.** Figure 5 shows a
scheme of an ASE apparatus. A solid or semi-solid sample is placed into a stainless steel extraction cell which is filled with solvent and heated (50–200°C) in an oven (Höfler et al., 1995a; Richter et al., 1996). The heating process generates solvent expansion and thus pressure in the extraction cell, typically in the region of 500–3000 psi. In order to prevent over-pressurisation of the cell, a static valve pulses open and closed automatically when the cell pressure exceeds the set point. The solvent that escapes during this venting is collected in a vial. A static extraction stage of about 5–10 min is followed by pumping fresh solvent through the system to rinse the sample and the tubing. All the solvent present in the system is then purged with a compressed gas, generally nitrogen (Richter et al., 1995, 1996; Ezzel et al., 1998; Levy, 1998), and the total solvent volume (extractant and rinsings) is collected in the vial. The Dionex apparatus offers the possibility of automation, and up to 24 samples can be sequentially extracted. Cells can be of different sizes: 1, 5, 11, 22 and 33 mL (Richter et al., 1995a; Richter, 1999). In this case, a restriction device. The extraction can be static or dynamic. In the dynamic mode, fresh solvent is continuously pumped through the sample at elevated temperature and pressure (Bautz et al., 1998). According to Fick’s first law of diffusion, the transfer rates are accelerated, leading to improved extraction efficiencies and reduced extraction times. Carbon dioxide can be used to flush the remaining solvent out of the extraction chamber (Ashraf-Khorassani et al., 1999). In this case, a wide range of cell sizes is available, from 0.2 to a few hundreds of millilitres. Furthermore, the possibility to perform either PSE or SFE using the same apparatus has obvious economical advantages.

Enhanced solvent extraction. An alternative approach to the above is to perform accelerated solvent extractions with a supercritical fluid extraction (SFE) apparatus (see Fig. 6; Li et al., 1998) in which the pressure is controlled by the restriction device. The extraction can be static or dynamic. In the dynamic mode, fresh solvent is continuously pumped through the sample at elevated temperature and pressure (Bautz et al., 1998). According to Fick’s first law of diffusion, the transfer rates are accelerated, leading to improved extraction efficiencies and reduced extraction times. Carbon dioxide can be used to flush the remaining solvent out of the extraction chamber (Ashraf-Khorassani et al., 1999). In this case, a wide range of cell sizes is available, from 0.2 to a few hundreds of millilitres. Furthermore, the possibility to perform either PSE or SFE using the same apparatus has obvious economical advantages.

Environmental applications

To date most of the published applications of ASE have been in the area of environmental research. The technique was applied successfully to the extraction of different pollutants from various environmental matrixes (Höfler et al., 1995b). After comparison with the corresponding reference methods, recoveries and precision were generally found equivalent, or even better. Most papers stress the shorter extraction times and lower solvent consumption of ASE in comparison to other conventional preparation techniques of environmental samples with the exception of SFE (Richter et al., 1995; Jensen et al., 1996; Kreisselmeyer and Dürbeck, 1997; Fisher et al., 1997). ASE and SFE can be considered as complementary, the first allowing the extraction of polar compounds, while the second is more selective for compounds of low or medium polarity. The ASE methodology is accepted in the US EPA SW-846 Method 3545A for selected priority pollutants from environmental matrixes (Richter et al., 1996; Levy, 1998).

Applications to the extraction of natural products

Very few applications of ASE have been published in the field of natural product research and the major contributions are summarised in Table 3.

An evaluation of ASE has been made for the extraction of various metabolites covering a large range of structures and polarities (curcuminoids, saponins, flavonolignans, terpenes) present in different vegetal matrixes such as leaves, roots, fruits, herbs and rhizomes (Benthin et al., 1999). Performances were compared to corresponding European Pharmacopoeia methods. Yields were found to be equivalent or even higher with ASE, with a reduction in the extraction time (especially when consecutive extractions with solvents of increasing polarity were made) and solvent consumption (by a factor from two to five), and with good reproducibility probably occasioned by the minimal sample handling required during the extraction procedure.

Curcuminoids. Three 6 min ASE cycles with methanol at 80°C were carried out on turmeric rhizomes (Curcuma xanthorrhiza). Almost all of the curcuminoids present in the sample were extracted during the first cycle, and the second and third extracts afforded only minimal additional curcuminoids. As the amount determined by the corresponding Pharmacopoeia method was much lower, the authors assumed that the latter method was not exhaustive.

Saponins. Horse chestnut (Aesculus hippocastanum) contains a saponin mixture called aescin. The Pharmacopoeia method for the extraction of this material involves 3 h defatting followed by 3.5 h of maceration in 65% methanol at room temperature and a 30 min reflux. A preliminary ASE defatting step (two cycles of 5 min each with dichloromethane at 100°C) was followed by extraction of saponins with 65% methanol at 100°C in two 6 min cycles. As previously mentioned, ASE recovery was higher than the corresponding Pharmacopoeia method.

Flavonolignans. Silybin is a flavonolignan contained in milk thistle fruit (Silybum marianum) and it possesses hepatoprotective properties. A single ASE treatment with 20 mL hexane (for defatting) followed by 5 min in methanol lead to the quantitative extraction of the flavonolignans. The amounts extracted were similar to those obtained using the traditional method which
Table 3. Application of ASE or PSE to natural product extraction

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Matrix</th>
<th>System</th>
<th>Extraction conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxanes (paclitaxel, baccatin III and 10-deacetylbaccatin III)</td>
<td><em>Taxus cuspidata</em> bark</td>
<td>ASE</td>
<td>Methanol:water (90:10); 10.13 MPa; 15 min; 150°C</td>
<td>Kawamura <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>Naphthodianthrones (hypericin)</td>
<td>St John’s wort herb, extracts, pharmaceuticals</td>
<td>ASE</td>
<td>Methanol; 100 bar; two cycles of 5 min with Extrelut in the cell; 40°C</td>
<td>Morf <em>et al.</em> (1998)</td>
</tr>
<tr>
<td>Naphthodianthrones</td>
<td>St John’s wort herb</td>
<td>ASE</td>
<td>Defatting with dichloromethane; 5 min; 100°C; followed by methanol extraction; three cycles of 5 min; 50–100°C</td>
<td>Benthin <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>Saponins</td>
<td>Horse chestnut seeds</td>
<td>ASE</td>
<td>Defatting with dichloromethane; two cycles of 5 min; 100°C; followed by extraction with 65% methanol; two cycles of 6 min; 100°C</td>
<td>Benthin <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>Terpenes</td>
<td>Thyme (<em>Thymus vulgaris</em>)</td>
<td>ASE</td>
<td>Hexane; one cycle of 5 min; 50°C; followed by dichloromethane; one cycle of 5 min; 50°C</td>
<td>Benthin <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>Flavonoolignans</td>
<td>Milk thistle fruit</td>
<td>ASE</td>
<td>Defatting with hexane (20 mL); one cycle; 100°C; followed by extraction with methanol (20 mL); one cycle of 5 min; 100°C</td>
<td>Benthin <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>Curuminoids</td>
<td>Turmeric rhizome</td>
<td>ASE</td>
<td>Methanol; one cycle of 6 min; 80°C</td>
<td>Benthin <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>Xanthones and flavonones</td>
<td>Osage orange tree (bark)</td>
<td>ASE</td>
<td>Dichloromethane; 13.8 MPa; three cycles of 5 min and 90 s purge; 40, 80 or 100°C</td>
<td>Da Costa <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>Cocaine and benzoylgonine</td>
<td><em>Erythroxylum coca</em> leaves</td>
<td>PSE</td>
<td>Methanol; 20 MPa; 1 mL/min; 10 min; 80°C</td>
<td>Brachet <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>Withanolides</td>
<td><em>Iochroma gesnerioides</em> leaves</td>
<td>PSE</td>
<td>Methanol:water (1 :1); 25 MPa; 0.5 mL/min; 10 min; 100°C</td>
<td>Kaufmann <em>et al.</em> (2001b)</td>
</tr>
</tbody>
</table>

Consists of a 4 h defatting step (100 mL petroleum ether) followed by Soxhlet extraction with 100 mL methanol. With ASE, the solvent consumption was reduced by a factor of 5.

**Terpenes.** Essential oil of thyme (*Thymus vulgaris*) was obtained by hydrodistillation and, simultaneously, the plant material was extracted by ASE. Thymol (the major component) was quantified by GC-FID and comparable results were demonstrated for both extraction methods. The ASE conditions were one 5 min cycle with hexane at 50°C, followed by one 5 min cycle with dichloromethane at the same temperature. Exhaustive extraction of the essential oil by hydrodistillation required 2 h.

**Taxanes.** ASE with methanol was used for the extraction of taxanes from the bark of *Taxus cuspidata* (Kawamura *et al.*, 1999) and permitted the extraction of paclitaxel, baccatin III and 10-deacetyl-baccatin III (10-DAB) in higher yields than could be obtained using classical SLE (0.10 MPa; 2 × 24 h; 22°C). For ASE various temperatures, pressures, extraction times and solvent composition were evaluated. The recoveries of taxanes were highest in the temperature range 100–150°C for paclitaxel and 120–150°C for 10-DAB. Degradation problems occurred at temperatures greater than 160°C. The yield hardly changed within the pressure range 1.01–20.27 MPa, when extraction was performed at 150°C for 15 min.

Particular attention has been given to ASE performed with water. Paclitaxel, although reported to be poorly soluble in this solvent, showed high recovery — some 50 times higher than that obtained with cold water extraction (22°C; 48 h) and five times higher than with hot water extraction (100°C; 1 h). Extraction at 10.13 MPa for 15 min at a temperature within the range 130–140°C for paclitaxel, and 120–130°C for 10-DAB, gave the best recoveries (Kawamura *et al.*, 1999). Various methanol:water ratios were also tested and it was shown that the higher the proportion of water the greater the recovery of overall extract from the bark: the highest yields of taxanes were obtained with a methanol:water ratio of 90:10.

**Naphthodianthrones.** ASE was applied to the recovery of naphthodianthrones (e.g. hypericin) from extracts and pharmaceuticals containing St John’s wort (*Hypericum perforatum*; Morf *et al.*, 1998; Benthin *et al.*, 1999). These compounds are known to show poor solubility in most organic solvents. The influence of different parameters on extraction efficiency, such as solvent, time, pressure, temperature and presence of filling material, was studied. The results obtained were compared with those of conventional methods (i.e. sonication and Soxhlet extraction) which are time consuming and require a preliminary pre-extraction step with dichloromethane followed by different treatments before a colorimetric measurement. A first ASE cycle with dichloromethane at 100°C was performed in order to
dealt the plant material, and this was followed by three cycles (5 min each) with methanol at a temperature ranging from 50 to 100°C. The total amounts extracted by ASE were greater than those obtained using classical methods, and the precision was around 1%.

**Xanthones and flavanones.** ASE, SFE and ASE have been compared with respect to the recovery of several phenolic compounds from bark of the Osage orange tree (*Maclura pomifera*; Da Costa et al., 1999). The ASE involved three cycles of 5 min static extraction at 13.8 MPa with 5 min equilibration time in between and a 90 s purge. Three temperatures (40, 80 and 100°C) were tested, and about 15 mL dichloromethane was used for each extraction. The ASE required 35 min compared with 45 min for SFE (using carbon dioxide modified with 20% methanol) or 48 h for solvent extraction. Both SFE and ASE extracted xanthones and flavanones from the plant material at similar or higher yields than those obtained with SLE.

**Alkaloids.** PSE was applied to the rapid extraction of cocaine and benzyloleugonine from coca leaves (Brachet et al., 2001). Several parameters, including the nature of the extracting solvent, the pressure, temperature, extraction time, addition of alkaline substances and the granular nature of the sample, were investigated in order to find the best extraction conditions. It was demonstrated that 10 min were sufficient to extract cocaine quantitatively at 80°C and 20 MPa. Furthermore, addition of an alkaline substance did not improve cocaine recovery, since degradation occurred during the extraction process.

**Steroids.** A PSE method was developed for the recovery of three withanolides (withaferin A, withanolide, and withaistanin) from the leaves of *Iochroma gesneroides* in the dynamic mode (Kaufmann et al., 2001b) and the performance of the method was compared to traditional Soxhlet extraction. The influence of the nature of the solvent, the flow rate, the pressure and the temperature of the extracting solvent, as well as the particle size of the plant material, on the recovery of analytes was investigated. A 1:1 mixture of methanol:water at a flow rate of 0.5 mL/min was found to be the most efficient extracting solvent and allowed a quantitative extraction in 10 min. PSE produced similar results to Soxhlet in terms of recovery, repeatability and selectivity; however, both total handling time and solvent consumption were dramatically reduced with PSE.

**CONCLUSIONS**

MAE and PSE are emerging as attractive alternatives to conventional extraction methods such as Soxhlet, percolation, digestion, extraction under reflux, sonication and in some cases steam distillation. Initially employed as a digestion method for different sample types such as environmental, biological and geological matrices, MAE is now widely accepted in analytical laboratories. The main advantage of MAE resides in the performance of the heating source. The high temperatures reached by microwave heating reduces dramatically both the extraction time and the volume of solvent required. PSE works according to the principle of SLE with elevated temperature and high pressures in order to keep the solvent in a liquid state. Enhanced diffusivity of the solvents leads to an increase in extraction speed and efficiency. With both MAE and PSE, recoveries of analytes and reproducibilities are improved and, therefore, both methods should be considered as interesting alternatives with the limitation that experimental conditions must be chosen in order to avoid possible thermal degradation. Finally, both the costs of the specialised equipment (especially PSE) may also influence the choice of the extraction technique (Camel, 2001).

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