Highly Efficient Microwave-Assisted and Ultrasonic-Assisted Extraction

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Abstract: Various extraction techniques were applied in the extraction of the biologically active constituents. The methods employed were traditional maceration extraction, microwave-assisted extraction(MAE), and ultrasound extraction (UE). Compared to the maceration extraction, MAE and UE methods provided high extraction yield, requiring short time frames and less labour. Microwave irradiation is a proven technique to be widely used in biotechnology.

Keywords: microwave-assisted extraction (MAE); ultrasonic-assisted extraction (UAE); microwave- assisted organic synthesis (MAOS)

1. Microwave-assisted extraction (MAE)

Among the emerging techniques, MAE, which had been succeeded in the extraction of organic compounds from solid samples such as soil, sediments, plants and animal tissues, needs less organic solvent and shorter extraction time than traditional extraction methods such as Soxhlet extraction. MAE, UAE and pressurized liquid extraction are relative newly developed extraction techniques designed to minimize the drawbacks of soxhlet extraction. Comparing with conventional extraction, MAE has some advantages in high efficiency and low cost. It can save time and money, and provide higher extraction yield.

High sample throughput is realized by a simultaneous sample treatment with microwave irradiation in an oven. Possible degradation of some compounds caused by contact with metals can be avoidable, because metal vessels are unnecessary. In addition, the MAE apparatus is relatively simple and reliable. The optimization of MAE procedures and comparison with other extraction techniques were reported in many articles. For MAE technique, water is a good solvent, as it possesses a high dielectric constant; hence it is characterized by a high ability to absorb microwave energy. Besides, a salt-out effect often improves the recovery in conventional extraction procedures. In this system, addition of ionic salts to sample solution could decrease the solubility of analyte and enhance the extracted quantity. Thus, saturated sodium chloride aqueous solution was added to the sample.

However, there are several disadvantages in using organic solvent for microwave-assisted extraction. First of all, most organic solvents may be dangerous to the operators and may result in environmental pollution because of the waste solvent disposal. Second, the organic solvent should generally be capable of absorbing the microwave energy. In some cases, solvents with low dielectric constants (low absorption of microwave energy) may be used, so a material may be added to absorb the energy and transfer it to the sample. Most of the time, this could be achieved by adding water to the the equipment, especially the extraction vessel. Some researches have reported the microwave-assisted sample matrix. Moreover, organic solvents at relative high temperature and pressure may corrode extraction of organic pollutants (e.g. organochlorine pesticides in medicinal plants) by using water as the solvent.

Improving the techniques used for the analysis of substance in solid matrices has been widely investigated to increase the recovery yields of analytes, to minimize waste solvents, and to shorten the analytical procedures and time. Emerging techniques, microwave-assisted extraction (MAE), pressurized fluid extraction (PFE) and supercritical fluid extraction (SFE) need a relatively short extraction time and a small amount of solvent. They sometimes give higher recovery yields of the analytes compared with conventional soxhlet extraction or saponification, because the extraction temperature can be increased higher than the boiling point of the extraction solvent under atmospheric pressure by performing the extraction procedures in closed pressure-resistant vessels.

Besides, there have been few investigations on the extraction, cleanup and determination of deca-BDE in waste electrical and electronic equipment. In this contribution, the microwave-assisted extraction coupled to headspace solid-phase microextraction (MAE-HS-SPME) and GC-MS spectrometry was investigated to develop a simple, rapid and solvent-free process to analyze essential oils in Magnolia bark. A microwave-assisted solvent extraction (MASE) method for the determination of methabenzthiazuron (MBT) in soil samples by HPLC-DAD (diode array detection) was evaluated[1].

Project supported by National Natural Science Foundation of China (No: 20872095).

2 . Microwave-assisted organic synthesis (MAOS)

MAOS has had a significant impact on organic and medicinal chemistry by dramatically shortening reaction times, producing cleaner product mixtures, and making high-energy transformations routine that might otherwise be avoided . Within the last five years, microwave technology has also been applied to reactions performed in a flowed format. Flowed chemical synthesis offers numerous advantages over traditional batch-reactor technology . Independent inlet streams allow reactive inter-mediates to be kept separate until brought together in miniscule amounts to react immediately; this rapidly depletes the starting materials and continuously physically moves the product away from the infusing stream. In batch reactors, product molecules form in the presence of a vast excess of starting materials that can lead to significant byproduct formation. Further, a moving synthesis platform allows for in-line analysis and instantaneous changes to reaction conditions for process optimization that can be automated readily. To gain the full advantage of working in flow, reactions should proceed very rapidly and ideally reach completion during the time in which the reactants reside in the flow tube. Microwave heating has been used to drive a wide variety of reactions to high levels of completion in a flowed format .

A microwave-assisted extraction (MAE) protocol and an efficient HPLC analysis method were first developed for the fast extraction and simultaneous determination of bisphenol F diglycidyl ether (Novolac glycidyl ether 2-Ring), Novolac glycidyl ether 3-Ring, Novolac glycidyl ether 4-Ring, Novolac glycidyl ether 5-Ring, Novolac glycidyl ether 6-Ring, bisphenol A diglycidyl ether, bisphenol A (2,3-dihydroxypropyl) glycidyl ether, bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether, bisphenol A bis(3-chloro-2-hydroxypropyl) ether, bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether in canned fish and meat. After being optimized in terms of solvents, microwave power and irradiation time, MAE was selected to carry out the extraction of ten target compounds. Analytes were purified by poly(styrene-co-divinylbenzene) SPE columns and determinated by HPLC-fluorescence detection. This proposed method was successfully applied to canned fish and meat, and the results acquired were in good accordance with the studies reported. Compared with the conventional liquid-liquid extraction and ultrasonic extraction, the optimized MAE approach gained the higher extraction efficiency .

3. Microwave-assisted combined with ultrasound extraction

Recently, modern extraction methods have been developed for the fast and efficient extraction of organic compounds from solid matrices, with microwave-assisted extraction (MAE) and ultrasonic extraction(UE) among the most promising for the extraction of natural products . MAE is the process of using microwave energy to heat solvents in contact with a sample in order to partition some chemical components from the matrix into the solvent. The benefits of UE are thought to be due mainly to the mechanic effects of acoustic cavitation. Both methods have recently demonstrated the potential to reduce extraction times significantly and increase extrac-tion yields in a number of studies on medicinal plants .

Resent research also showed that the total amounts of extracted phenolics and flavonoids were determined, and the effectiveness of the methods compared. MAE was very rapid but led to the extraction of a large amount of non-phenolic and non-flavonoid material. UE gave the highest percentage of extracted phenolics. Compared to the maceration extraction, MAE and UE methods provided high extraction yield, requiring short timeframes and lesslabour. UE was shown to be the most efficient method based on yield, extraction time and selectivity.MAE of bioactive phenolics and flavonoids from poplar type propolis was found to be a very fast extraction method, compared to maceration and even UE. However, the extraction selectivity was low, with significant amounts of unwanted wax was found to have been extracted. In addition, longer irradiation times resulted in a decrease in the percentage of extracted active compo-nents, presumably owing to degradation processes. UE has been shown to be the most efficient extraction method, taking into consideration yield, short extraction time and high selectivity. saponification, sonication, and Soxhlet extraction, are also given in this report. The techniques gave comparable results with the values obtained by other extraction techniques and the certified values in the samples. However, the observed concentration values of mono- and dichlorinated biphenyls varied depending on the extraction temperature.

The aim of the preliminary study is to compare the effectiveness of the extraction of bioactive components from poplar propolis (phenolics, flavones/flavonols and flavanones/dihydroflavonols) using the maceration method, MAE and UE. Scientists have chosen to quantify groups of active compounds with the same or similar chemical structure rather than individual substances. Recently, it has been demonstrated that the concentration of such compound groups in propolis extracts correlates much better with the levels of antibacterial activity and is more informative than the concentration of individual components.

Microwave-assisted extraction (MAE) and microwave-assisted hydrolysis (MAAH) were developed for the sample preparation of guava leaves prior to GC determination of quercetin and its glycosides. Ethanol was selected as the solvent. To evaluate the merits of the developed MAE and MAAH methods, the quercetin

978-1-935068-37-2 © 2010 SciRes.

yields obtained were compared with those obtained by reflux heating. The results showed that can be seen that MAE and MAAH gave the highest quercetin yield within minutes, whereas reflux heating took hours with even lower quercetin yield. It is considerable that the main reason for these diverse results is due to the fundamental difference between microwave heating and conventional heating. The rotation of molecules in an attempt to align themselves with an applied microwave field and the strong interaction of ions with the electrical field of microwaves produce specific effects: superheating, mass heating and fast heating which cannot be obtained by conventional heating[2].

4. Microwave-assisted extraction(MAE) applicated to biotechnology

One application of biotechnology is to use human genetic information to discover, develop, manufacture, and commercialize biotherapeutics. Recombinant proteins can be produced in large quantities at high purity. High-throughput proteomic analysis is at the heart of the biotechnology research and development process, and the industry is constantly striving to streamline and automate the analytical processes involved. Microwave-assisted proteomics has recently emerged as a tool for increasing the bio-catalysis of several processes including tryptic digestions lipase selectivities, identification of metal-catalyzed oxidation sites on proteins, identification of protein N- and C-termini and enzyme catalyzed N-linked deglycosylation. We may explore the above mentioned methods, and describe our experiences evaluating microwave-technology for other common proteomic protocols including: removal of N-terminal pyroglutamyl for antibody characterization, beta elimination and Michael addition for identification of phosphorylation sites on recombinant proteins and enzyme mediated O-linked deglycosylation[3].

Microwave irradiation is a proven technique to reduce post-mortem protein degradation. In curtail post-mortem protein degradation, a number of methods have been employed, including the use of transgenic mice lacking carboxypeptidase activity, focused microwave irradiation for animal sacrifice, and rapid post-sacrificial microwave irradiation. Microwave irradiation heat deactivates endogenous proteases and arrests post-mortem protein degradation, resulting in a clean neuro-peptide sample for MS analysis. However, the equipment employed in focused microwave irradiation sacrifice is expensive and requires the restraint of the animal during sacrifice. In contrast, post-sacrifice microwave irradiation employs an inexpensive household microwave. However, this technique still requires the time and animals to develop consistent protocols. On the basis of experiments employing protease heat deactivation, scientists had developed a neuropeptide extraction protocol which combined snap-freezing, cryostat dissection, and a boiling extraction buffer to curtail protein degradation without the use of microwave irradiation[4].

A simple analytical procedure for the simultaneous determination of eight endogenous steroids in aquatic molluscs by solid-phase extraction (SPE) and gas chromatography/mass spectrometry (GC–MS) has been developed. After a microwave-assisted extraction, samples were further extracted and purified using two successive SPE (EnviChrom-P and NH_2)cartridges[5,6].

Solid-phase strategies speed up the production of both short- and long-sequence peptides compared with solution methodologies. Therefore, solid-phase peptide synthesis (SPPS), proposed by Merrifield in the early 1960s, contributed to the 'Peptide Revolution' in the fields of diagnostics, and drug and vaccine development. Since then, peptide chemistry research has aimed to optimize these synthetic procedures, focusing on areas such as amide bond formation (the coupling step), solid supports and automation. Particular attention was devoted to the environmental impact of SPPS: the requirement for large amounts of organic solvents meant high costs for industrial peptide manufacturing that needed to be reduced. SPPS, alone or in hybrid technologies, has become strategic for the production of peptides as active pharmaceutical ingredients on a commercial scale[7].

Differentiation of the physiological role of the melanocortin receptor MC5R from that of other melanocortin receptors will require development of high affinity and selective antagonists. To date, a few synthetic antagonist ligands active at hMC5 receptor are available, but most do not have appreciable selectivity. With the aim to gain more potent and selective antagonists for the MC5R ligands, we have designed, synthesized, and pharmacologically characterized a series of alkyl-thioaryl-bridged macrocyclic peptide analogues derived from MT-II and SHU9119. These 20-membered macrocycles were synthesized by a tandem combination using solid phase peptide synthesis and microwave-assisted reactions[8].

p53 is widely used as an indicator of tumor aggression and progression. Fixation methods especially formaldehyde based fixation may mask the immunohistochemical detection of p53 protein but antigen retrieval methods enhance the inmmunohistochemical detection of p53 protein by remodification of protein structure. Studies was designed to evaluate the efficacy of different fixatives, of microwaving and microwave pretreatment method to retrieve p53 immunoreactivity in paraffin-embedded non-lesioned (adjacent normal tissue) human skin samples or pathological human skin samples diagnosed as basal cell carcinoma. Optimal results were obtained using samples fixed in alcohol either at room temperature or in microwave oven[9].

The detection of glycoproteins on SDS-PAGE gels is a very challenging task as glycan moieties can inhibit the protein-dye interaction or protein-silver reaction

slowing down or even completely preventing the staining process. The staining procedures were performed with and without the assistance of microwave irradiation. Microwave treatment resulted in comparable band intensities and sensitivities as obtained by the original staining protocols, but staining duration was significantly reduced. PMF analysis by MALDI mass spectrometry was not affected by the microwave treatment. It was found that the total number of detected tryptic peptides has increased when applying microwave irradiation during the staining process[10].

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978-1-935068-37-2