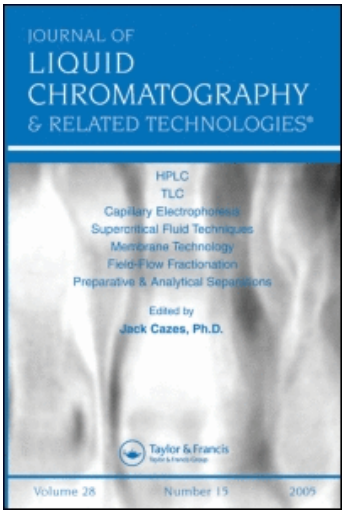


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Comparison of the Different Extraction Methods of Flavonoids in *Epimedium Koreamum Nakai* by HPLC-DAD-ESI-MS^n

Zhongying Liu[a]; Lan Ding[a]; Hanqi Zhang[a]; Xiuli Hu[b]; Fengquan Bu[b]
[a] College of Chemistry, Jilin University, Changchun, P.R. China [b] School of Pharmacy, Jilin University, Changchun, P.R. China

To cite this Article Liu, Zhongying , Ding, Lan , Zhang, Hanqi , Hu, Xiuli and Bu, Fengquan(2006) 'Comparison of the Different Extraction Methods of Flavonoids in *Epimedium Koreamum Nakai* by HPLC-DAD-ESI-MS^n', Journal of Liquid Chromatography & Related Technologies, 29: 5, 719 — 731
To link to this Article: DOI: 10.1080/10826070500509280
URL: http://dx.doi.org/10.1080/10826070500509280

Journal of Liquid Chromatography & Related Technologies®, 29: 719–731, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070500509280

Comparison of the Different Extraction Methods of Flavonoids in *Epimedium Koreamum Nakai* by HPLC-DAD-ESI-MS^n

Zhongying Liu, Lan Ding, and Hanqi Zhang
College of Chemistry, Jilin University, Changchun, P.R. China

Xiuli Hu and Fengquan Bu
School of Pharmacy, Jilin University, Changchun, P.R. China

Abstract: In the present paper, the flavonoids in *Epimedium Koreamum Nakai* have been extracted by atmospheric pressure microwave assisted extraction (AMAE), pressurized microwave assisted extraction (PMAE), ultrasonic extraction (UE), and reflux extraction (RE), and the extracts have analyzed by HPLC-DAD-ESI-MS^n. As a result, the four flavonoids in the extracts have been identified as epimedin A, epimedin B, epimedin C, and icariin. Also, the influence of extraction parameters on the extraction yields of epimedin A, epimedin B, epimedin C, and icariin has been evaluated by comparison with the peak areas of HPLC-DAD spectra in the different extraction methods. The experimental results demonstrate that the advantage of PMAE and AMAE over conventional RE is validated, the extraction time is dramatically reduced, and the yields of flavonoids are effective improved. Although the UE method can be carried out within a short time, the extraction yields of flavonoids are lower compared with other extraction methods.

Keywords: Flavonoids, *Epimedium Koreamum Nakai*, Extraction method, Comparison, HPLC-DAD-ESI-MS^n

INTRODUCTION

The extraction process is the key step in the analyses of medicinal plants, so the optimization of the extraction processes for different medicinal plants is

Address correspondence to Hanqi Zhang, College of Chemistry, Jilin University, Changchun 130012, P.R. China. E-mail: analchem@jlu.edu.cn

very important. As is well known, both the Soxhlet extraction (SE) and reflux extraction (RE) are traditional extraction methods of chemical components in medicinal plants and are labor intensive, time consuming, and require excessive sample amounts. In recent years, some novel extraction techniques have been developed, for example, microwave-assisted extraction (MAE)[1] and ultrasonic extraction (UE),[2–5] in which 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 sample matrix into the solvent; the rapid heating of the sample mixture is the main advantage of the MAE technique. More recently, the MAE technique has been applied to extract chemical components from medicinal plants.[6–10] Two types of microwave heating systems are commercially available for the analytical laboratories: the closed- and the open-vessel systems. The former is usually called pressurized microwave assisted extraction (PMAE), in which many chemical components in medicinal plants have been extracted, such as puerarin from the Radix puerariae[11] and flavonoids from *Acanthopanax Senticosus* Harms.[12] By using closed vessels, the extraction process can be performed at high pressure, and accelerated with increasing the temperature in the system, and a few of the extracts can be simultaneously prepared. However, many side reactions may occur at high pressure. The latter, is also named atmospheric pressure microwave-assisted extraction (AMAE), in which the sample is usually immersed in the microwave absorbing solvent in open vessels, and then irradiated by means of the microwave energy until completion of the extraction process. The AMAE technique has been applied to the extraction of chemical components in medicinal plants and is suitable for preparing large amounts of sample.[13,14] It has also been reported, that the extraction of flavonoids in Flos Sophorae at atmospheric pressure has been performed by using a modified household microwave oven,[15] and higher extraction efficiency was obtained.

The UE of chemical components in medicinal plants has been found in the literature as early as the 1950's.[16] Recently, there have been a number of reports on the application of ultrasound in phytochemistry, such as the extractions of alkaloids, flavonoids, polysaccharides, proteins, and essential oils from various parts of plants.[17–22] It has been found, that ultrasound can aid the extraction of chemical components in a number of plants by significantly reducing extraction times and increasing extraction yields. The effects of ultrasonic energy on the cell walls of plants was described by Wu.[19] It has been suggested, that the improvement of UE is mainly due to the mechanical effects of acoustic cavitation, which enhances both solvent penetration into the plant material and the intracellular product release, by disrupting the cell walls. Another advantage of UE is that decomposition of the numerous components in the plants can be avoided.

Epimedium Koreamum Nakai is widely distributed in Northern China and used as a tonic and antirheumatic in Traditional Chinese medicine.[23] Generally, the flavonoids, i.e., icariin, epimedin A, epimedin B, and

epimedin C, have been reported as the main bioactive components in *Epimedium Koreamum Nakai*.[24,25]

In this report, the influence of extraction parameters on the extraction yields of epimedins A, B, C, and icariin in *Epimedium Koreamum Nakai* was investigated by the PMAE, AMAE, UE, and RE methods. The extracts obtained by these different methods were analysed by HPLC-DAD-ESI-MS^n.

EXPERIMENTAL

Instrumentation

The HPLC analyses were carried out on a Waters liquid chromatograph connected with a 2690 Alliance Waters quaternary pump. The detection was performed with a Waters 996 photodiode array detector (DAD) working in the range of 200–650 nm (Milford, MA, USA). The chromatographic data were recorded and processed by means of the Waters Millennium 2015 software.

For electrospray mass spectrometry (ESI-MS) analyses, a Finnigan MAT LCQ ion trap mass spectrometer (Finnigan-MAT, San Jose, CA, USA) with an electrospray source was used. The spray voltage was set to 50 kV. All mass spectra were acquired at a capillary temperature of 200°C.

The WR-E microwave sample preparation system with a pressure control system is from the Meichengkemao Group, (Beijing, China). The extraction vessel consists of a vessel body and a liner vessel. A modified household microwave oven (National, Japan) with the maximum power of 700 W was used for MAE at atmospheric pressure. The UE instrument with an ultrasonication bath was from the Shanghai Ultrasonication Instrument Factory (Shanghai, China).

Chemicals and Plant Materials

Ethanol, of analytical grade, was obtained from the Beijing Chemical Factory (Beijing, China). Acetonitrile and acetic acid (HPLC grade) used in the HPLC analyses were purchased from Fisher Company (UK). The standard of icariin was purchased from the Chinese Authenticating Institute of Material and Biological Products (Beijing, China). Pure water was obtained by means of the Milli-Q water purification system (Millipore Corporation, USA).

The dried leaves of *Epimedium Koreamum Nakai* collected in Fusong City (Jilin Province, China), were crushed and passed through a 0.9 mm sieve, then immersed in petroleum ether (b.p. 60–90°C) overnight at room temperature, in order to remove chlorophyll. After filtration, the residue was given an airing at room temperature, in order to remove the petroleum ether. Dried powder was obtained.

Extraction Procedures

Reflux Extraction (RE)

A sample powder (2.5000 g) was accurately weighed and then put in the distilling flask, fitted with a water cooling condenser, in which 200 mL of 40% ethanol used as the extraction solvent was added. After being heated and refluxed for a given time, the extract was filtrated and transferred into a 250 mL volumetric flask. The solvent was used to rinse the distilling flask and the sediment three times. The rinsed solvent was also added into the flask and then the solvent was added to the graduation of the flask.

Pressurized Microwave Assisted Extraction (PMAE)

In PMAE, the aqueous solution of ethanol was used as the extraction solvent. A sample powder of 0.5000 g was accurately weighed, then turned into the liner vessel of the digestion vessel, and immersed by 20 mL of the extraction solvent. The liner vessel was put into the vessel body. The control vessel was connected to the pressure control device. The closed sample vessels and the control vessel were put into the microwave preparation system. Then, the pressure in the digestion vessel was gradually increased until it reached the preset pressure. The extraction was continuously carried out under the preset pressure for a given time, which was performed intermittently, i.e., irradiation-cooling-irradiation for 2 to 21 min; the irradiation time was maintained for 20 s, during which 5 s was taken for the cooling of the sample between two irradiation processes. When the extraction was finished, the samples were allowed to cool down to room temperature, then, the extraction solution was filtrated and transferred into a 50 mL flask. The solvent was used to rinse the liner vessel and the sediment three times. The rinsed solvent was also added into the flask and then the solvent was added to the mark.

Atmospheric Pressure Microwave Assisted Extraction (AMAE)

In AMAE, the aqueous solution of ethanol was used as the extraction solvent. A sample powder of 0.5000 g was accurately weighed and put into a distillation flask (250 mL) to which 40 mL of the extraction solvent was added; this was performed intermittently, i.e., irradiation-cooling-irradiation for 2 to 30 min. The extraction time was maintained for 8 s, and then, the sample solution was cooled for 14 s between the two irradiation processes. The extract was cooled, filtrated, and then transferred into a 50 mL volumetric flask. The solvent was used to rinse the distilling flask and the sediment three times. The rinsed solvent was also added to the flask, then the solvent was added to the mark.

Ultrasonic Extraction (UE)

A sample powder (0.5000 g) was accurately weighed, and then put into a 50 mL flask, into which 40 mL of 40% ethanol was added. The sample was extracted by an ultrasonic method for 5 to 50 min. After filtration, the extract was transferred into a 50 mL flask. The solvent was used to rinse the distilling flask and the sediment three times. The rinsed solvent was also added to the flask up to the mark.

All experiments were performed in triplicate.

HPLC-DAD-ESI-MS^n Analyses

The extracts obtained from the different extraction methods, i.e., PMAE, AMAE, UE, and RE, were centrifuged and filtered through a 0.45 μm microporous membrane and then analyzed by HPLC using a Zorbax ODS C_{18} (5 μm, 250 × 4.6 mm, Dupont) column, and the injection volume was 10 μL, the mobile phase was acetonitrile : water (0.5% acetic acid) (30 : 70, V/V) and the flow rate was 0.8 mL/min. The eluates were monitored by using a DAD detector at 270 nm. The four components were separated and identified by the retention time in HPLC and mass spectrometry data in ESI-MS^n, which were in agreement with that reported in the reference.

RESULTS AND DISCUSSION

HPLC-DAD-ESI-MS^n Investigation of the Flavonoids in *Epimedium Koreamum Nakai* Extracted by PMAE

The flavonoids in *Epimedium Koreamum Nakai* have been extracted by PMAE, which have been analyzed by HPLC-DAD-ESI-MS^n. HPLC results are given in Figure 1, and the retention time (RT) in HPLC and ESI-MS^n data of constituents 1, 2, 3, and 4 are given in Table 1. The experimental

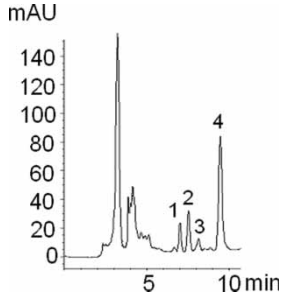


Figure 1. HPLC curve of the flavonoids extracted from *Epimedium Koreamum Nakai*.

Table 1. Retention time in HPLC and ESI-MS^n data of constituents 1, 2, 3 and 4

Peak	RT	ESI-MS^n data								Compound
1	7.01	839 m/z	$\xrightarrow[MS^2]{-162}$	677 m/z	$\xrightarrow[MS^2]{-146}$	531 m/z	$\xrightarrow[MS^3]{-162}$	369 m/z		Epimedin A
2	7.53	809 m/z	$\xrightarrow[MS^2]{-132}$	677 m/z	$\xrightarrow[MS^2]{-146}$	531 m/z	$\xrightarrow[MS^3]{-162}$	369 m/z		Epimedin B
3	8.13	823 m/z	$\xrightarrow[MS^2]{-146}$	677 m/z	$\xrightarrow[MS^2]{-146}$	531 m/z	$\xrightarrow[MS^3]{-162}$	369 m/z		Epimedin C
4	9.46	677 m/z	$\xrightarrow[MS^2]{-146}$	531 m/z	$\xrightarrow[MS^3]{-162}$	369 m/z				Icariin

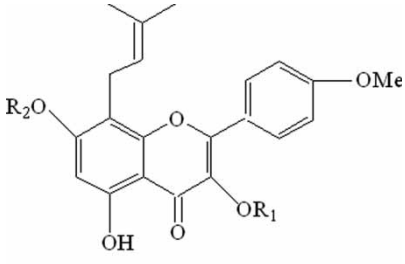
results demonstrate that the four constituents have been found in HPLC, which have been identified and elucidated as epimedin A, epimedin B, epimedin C, and icariin, respectively, according to the data of the icariin standard and the literatures;[24–26] their structures are shown in Figure 2.

The Influence of Ethanol Concentration on the Extraction Yields

As is well known, the MAE yield depends on both the polarity of the extraction solvent and the solubility of the plant constituents in the solvent. In the present paper, the ethanol and distilled water were used as the extraction solvents in the MAE. When the extraction pressure is 300 kPa and extraction time is 3 min, the effect of ethanol concentration on the yields of the four flavonoids, i.e., epimedin A, epimedin B, epimedin C, and icariin, are shown in Figure 3. The experimental results demonstrate that the highest yields are obtained by using 40% ethanol as extraction solvent for the four compounds. In order to compare the extraction yields of various extraction methods, 40% (V/V) ethanol was used as the extraction solvent in AMAE, UE, and RE.

The Influence of Extraction Pressure on the Extraction Yields

The extraction yield depends on both the extraction efficiency and chemical change of the target compound. In the extraction process, the target



R_1	R_2	Compound
rha —(2)— glc	glc	Epimedin A
rha —(2)— xyl	glc	Epimedin B
rha —(2)— rha	glc	Epimedin C
rha	glc	Icariin

Figure 2. The molecular structures of epimedin A, epimedin B, epimedin C, and icariin in *Epimedium Koreamum Nakai*.

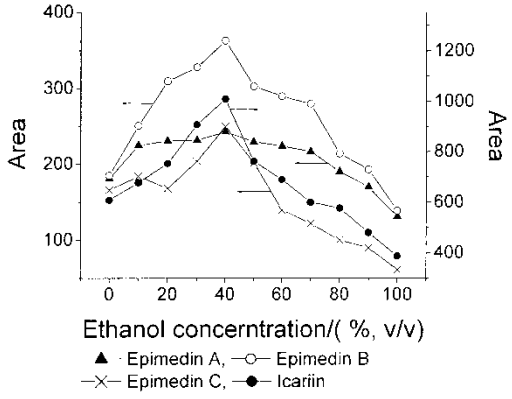


Figure 3. The influence of ethanol concentration on the yields.

compound is first transferred into the extraction solvent. The amount of the target compound transferred into the solvent is defined as the extraction efficiency. After the target compound is transferred into the extraction solvent, chemical changes may occur for the target compound at some experimental condition, which can directly influence the final extraction yield of target compound. In PMAE, the sample powder was extracted with 40% ethanol for 3 min, the influence of extraction pressure on extraction yield was shown in Figure 4. The experimental results demonstrate that the extraction yield of icariin increases with the enhancement of the extraction pressure when the processing pressure is in the range of 100 kPa to 600 kPa, and then, the extraction yield is not significantly changed from 600 kPa to 1000 kPa. The extraction yields of epimedin A, epimedin B, and epimedin C increase with the enhancement of the extraction pressure

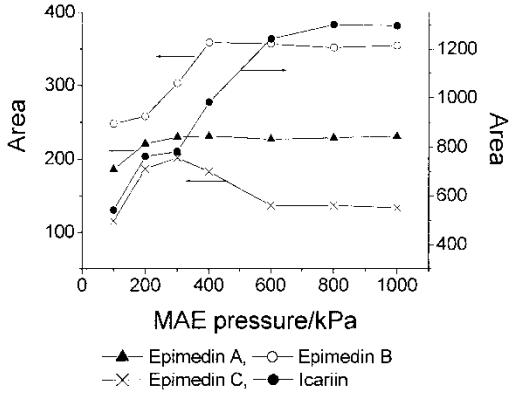


Figure 4. The influence of the extraction pressure on the yields.

up to 300 kPa, 400 kPa, and 300 kPa, respectively, and then, the extraction yields of epimedin A, epimedin B, remained constant. On the contrary, the extraction yield of epimedin C significantly decreases with the enhancing of the extraction pressure when the processing pressure is in the range of 300 kPa to 600 kPa, and then, the extraction yield is not significantly changed from 600 kPa to 1000 kPa. From above results, WE can deduce that epimedin C in extract may be partly decomposed into icariin by the loss of a rhamnose. The experimental results show that PMAE can accelerate the transfer of target compounds into the extraction solvent, but on the other hand, the chemical change of some compounds in this process may occur.

Influence of the Extraction Time on the Extraction Yields

In order to study the influence of the extraction time on the extraction yields, PMAE (at 300 kPa), AMAE, and UE were performed at different times by using 40% (V/V) ethanol as the extraction solvent. The effects of the extraction time on the extraction yields of epimedin A, epimedin B, epimedin C, and icariin have been investigated and the results are shown in Figure 5(a), (b), and (c), respectively. The experimental results indicate that the values of the extraction time for the four components in the AMAE process (Figure 5(a)) are longer than those in PMAE (Figure 5(b)), respectively, and compared with the AMAE and PMAE, the extraction time in UE (Figure 5(c)) is the longest for each component. The extraction yield obtained by PMAE is higher than that obtained by AMAE or UE, which is due to the extraction process is accelerating at higher pressure.

In AMAE, the extraction yields of epimedin A, epimedin B, and epimedin C increase with the increase of the extraction time from 2 to 14 min, and do not change from 14 to 30 min. Meanwhile, the extraction yield of icariin increases with the increase of extraction time from 2 to 22 min.

In PMAE, the extraction yields of epimedin A and epimedin B increase with the increase of the extraction time from 2 to 6 min and do not significantly change from 6 to 21 min. The extraction yield of epimedin C increases with the increase of the extraction time from 2 to 4 min and then decreases from 4 to 5 min, and does not significantly change from 5 to 21 min. With the increase of MAE time, the extraction yield of icariin increases before 15 min, and then is unchanged.

As mentioned above, two possibilities lead to these results, one is that the extraction of epimedin A and epimedin B have been completed at 6 min, and another is that the extraction efficiency of epimedin A and epimedin B increase with the increase of MAE time after 6 min, especially, for epimedin C, the extraction yield decreases from 4 to 5 min, with no change from 5 to 21 min. However, they are partly decomposed into icariin by the loss of a glucose, a xylose, or a rhamnose. As a result, the yield of

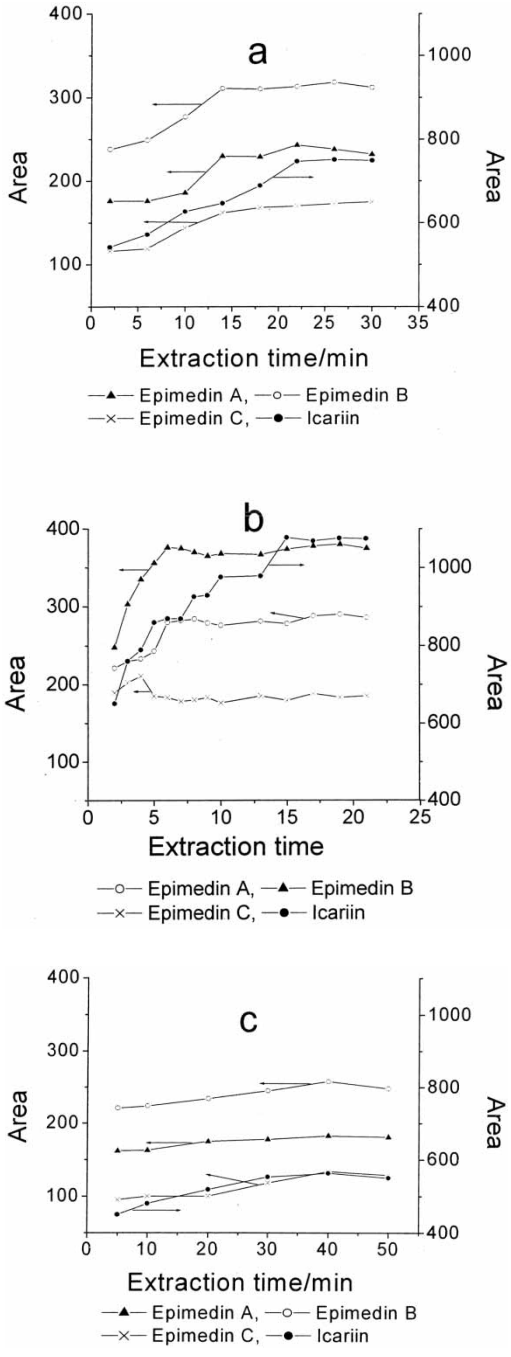


Figure 5. Influence of the extraction time on the yields, (a): AMAE; (b): PMAE; (c): UE.

Table 2. Comparison of the extraction yields of PMAE, AMAE, UE and RE (mAU × min), mean ± SD, n = 6

Extraction method	Peak area			
	Epimedin A	Epimedin B	Epimedin C	Icariin
AMAE	243 ± 21	311 ± 25	162 ± 16	747 ± 27
PMAE	280 ± 27[a]	375 ± 33[a]	183 ± 14	870 ± 35[a]
UE	183 ± 20[a]	258 ± 18[a]	134 ± 11[a]	553 ± 23[a]
RE	241 ± 19	277 ± 27	151 ± 13	766 ± 26

[a]Comparing with that obtained by RE, $p < 0.01$.

epimedin A, epimedin B, and epimedin C do not change and the extraction yield of icariin increases with the increase of MAE time before 15 min.

Epimedin A, epimedin B, epimedin C, and icariin are completely extracted by using UE for 40 min. The experimental results demonstrate that the UE time is longer than the AMAE time, but shorter than the reflux extraction time of 3 h.

The Comparison of the Extraction Yields Obtained by the Different Extraction Methods

The extraction yields obtained by PMAE (300 kPa, 6 min), AMAE (22 min), UE (40 min), and RE (3 h) have been compared by means of the analyses of variance with the Student's t-test. The statistics analyses results show that the extraction yields of epimedin A, epimedin B, epimedin C, and icariin obtained by PMAE (40% ethanol, 300 kPa, 3 min) are highest, and the lowest extraction yield is obtained by using UE (Table 2).

CONCLUSIONS

In this study, the four constituents (epimedin A, epimedin B, epimedin C, and icariin) extracted from *Epimedium* were identified by HPLC-UV-MS and determined by HPLC-UV. Comparing the yields of the four constituents obtained by using pressurized MAE, atmospheric pressure MAE, UE, and conventional reflux extraction methods, the advantage of pressurized MAE and atmospheric pressure MAE over conventional reflux extraction, is validated in that the extraction time is dramatically reduced and the yields of effective constituents extracted from *Epimedium* are improved. The other advantage observed from the MAE is a reduced volume of solvents required. Although, UE should be carried out within a short time and minimum of solvent, the extraction yields of effective constituents are lower.

ACKNOWLEDGMENT

This work was supported by the Natural Science Foundation of Jilin Province, China (Grant number: 20030551-7).

REFERENCES

1. Eskilsson, C.S.; Bjorklund, E. Analytical scale microwave-assisted extraction. J. Chromatogr. A **2000**, *902*, 227–250.
2. Martens, D.; Gfrerer, M.; Wenzl, T.; Zhang, A.; Gawik, B.M.; Schramm, K.-W.; Lankmayr, E.; Kettrup, A. Comparison of different extraction techniques for the determination of polychlorinated organic compounds in sediment. Anal. Bioanal. Chem. **2002**, *372*, 562–568.
3. Huie, C.W. A review of modern sample preparation techniques for the extraction and analysis of medicinal plants. Anal. Bioanal. Chem. **2002**, *373*, 23–30.
4. Vinatoru, M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrason. Sonochem **2001**, *8*, 303–313.
5. Guo, M.; Song, F. Ultrasonic extraction and determination of total saponins from leaves of *Acanthopanax Senticosus* Harms. Chinese J. Anal. Chem. **2002**, *30*, 250.
6. Luque-Garcia, J.L.; Luque de Castro, M.D. Water Soxhlet extraction assisted by focused microwaves: A clean approach. Anal. Chem. **2001**, *73*, 5903–5908.
7. Shu, Y.Y.; Ko, M.Y.; Chang, Y.S. Microwave-assisted extraction of ginsenosides from ginseng root. Microchem. J. **2003**, *74*, 131–139.
8. Stashenko, E.E.; Jaramillo, B.E.; Martinez, J.R. Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N.E. Brown, grown in Colombia, and evaluation of its in vitro antioxidant activity. J. Chromatogr. A **2004**, *1025*, 93–103.
9. Pan, X.J.; Hiu, G.G.; Liu, H.Z. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. Chem. Eng. Proc. **2003**, *42*, 129–133.
10. Dai, J.; Yaylayan, V.A.; Raghaban, G.S.V. Influence of operating parameters on the use of the microwave-assisted process (MAP) for the extraction of under atmospheric pressure conditions. J. Agric. Food Chem. **2001**, *49*, 4584–4588.
11. Guo, Z.; Jin, Q.; Fan, G.; Duan, Y.; Qin, C.; Wen, M. Microwave-assisted extraction of effective constituents from a Chinese herbal medicine Radix puerariae. Anal. Chim. Acta **2001**, *436*, 41–47.
12. Liu, Z.; Sun, J.; Li, M.; Yan, G.; Zhang, H. Microwave assisted extraction of flavonoids from Acanthopanax Senticosus Harms. Chinese J. Anal. Chem. **2004**, *32*, 360–363.
13. Li, H.; Chen, B.; Zhang, Z.; Yao, S. Focused microwave-assisted solvent extraction and HPLC determination of effective constituents in Eucommia ulmodies Oliv. (E. ulmodies). Talanta **2004**, *63*, 659–665.
14. Kwon, J.H.; Belanger, J.M.R.; Pare, J.R.J.; Yaylayan, V.A. Application of the microwave-assisted process (MAP^{TM}) to the fast extraction of ginseng saponins. Food Res. Intl. **2003**, *36*, 491–498.
15. Li, M.; You, J.; Liu, Z.; Zhang, H. Microwave-assisted dynamic extraction of flavonoids from *Flos Sophaoae*. Chem. J. Chinese Univ. **2004**, *25*, 850–852.
16. Schmall, A.; Brau-Rundschau, S. The application of ultrasonics in the brewery. Chem. Abstr. **1954**, *47*, 2932d.

17. Sargenti, S.R.; Vichnewski, W. Sonication and liquid chromatography as a rapid technique for extraction and fractionation of plant material. Phytochem. Anal. **2000**, *11*, 69–73.
18. Paniwnyk, L.; Beaufoy, E.; Lorimer, J.P.; Mason, T.J. The extraction of rutin from flower buds of Sophora japonica, Ultrason. Sonochem. **2001**, *8*, 299–301.
19. Wu, J.; Lin, L.; Chau, F. Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells. Ultrason. Sonochem. **2001**, *8*, 347–352.
20. Vinatoru, M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrason. Sonochem. **2001**, *8*, 303–313.
21. Hromadkova, Z.; Ebringerova, A.; Valachovic, P. Comparison of classical and ultrasound-assisted extraction of polysaccharides from Salvia officinalis L. Ultrason. Sonochem. **1999**, *5*, 163–168.
22. Vila, D.H.; Mira, F.J.H.; Lucena, R.B.; Recamales, M.A.F. Optimization of an extraction method of aroma compounds in white wine using ultrasound. Talanta **1999**, *50*, 413–438.
23. Editorial Committee of the Pharmacopoeia of People's Republic of China. *The Pharmacopoeia of People's Republic of China (Part 2)*; Chemical Industry Press: Beijing, 2000, 621.
24. Yuan, J.; Gong, Y.; Ju, P.; Kong, L. HPLC-MS^2 analysis of chemical constituents in *Epimedium koreanum*. Chinese Traditional and Herbal Drugs **2004**, *35*, 371–374.
25. Huang, C.H.; Zhao, L.H.; Mei, L.H.; Fu, H. Studies on the HPLC fingerprints of Epimedium brevicornum crude drugs. Chin. J. Nat. Med. **2003**, *1*, 146–148.
26. Dou, J.P.; Liu, Z.Q.; Liu, S.Y. Studies on icariin by electrospray ionization mass spectrometry. Chem. J. Chinese Univ. **2004**, *25*, 837–840.

Received November 1, 2005
Accepted December 3, 2005
Manuscript 6747