We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

6,600 Open access books available
177,000 International authors and editors
195M Downloads

154 Countries delivered to
TOP 1% Our authors are among the most cited scientists
12.2% Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Chapter 5


Pin-Huei Chen, Pai-Shan Chen and Shang-Da Huang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55971

1. Introduction

A chromatographic method combined with mass spectrometry is the key technique used in analysis of trace-level compounds present in complex matrices. However, success depends on the enrichment and extraction of target analytes from the matrix. This study investigated the development of a microextraction technique for use in the analysis of pesticides present at trace levels in field water samples and vegetable matter.

The most common extraction techniques used in environmental analysis are liquid–liquid extraction (LLE) [1, 2] and solid-phase extraction (SPE) [3, 4], both of which are time-consuming and use large volumes of samples. As a result, much attention is being paid to the development of more efficient and environmentally friendly extraction techniques, such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME). SPME, a solvent-free technique, was developed by Arthur and Pawlizyn [5]; however, the fiber is expensive and fragile, and the problem of sample carry-over cannot always be eliminated [4, 6-8]. LPME, which was introduced by Cantwell and co-workers [9], minimizes solvent usage and solvent variation; this technique is of interest to many analysts. Single-droplet microextraction (SDME) [10-13], solvent-bar microextraction (SBME) [14, 15] and hollow fiber-LPME (HF-LPME) [16-19] have been developed in the past few years but longer extraction times are required to obtain good extraction efficiencies. Efforts to overcome these limitations led to the development of dispersive liquid–liquid microextraction (DLLME) with the advantages of short extraction times, ease of operation, and small amounts of solvents used [20-22]. In DLLME, a water-immiscible extraction solvent, which is dissolved in a water-miscible dispersive solvent,
is introduced rapidly by syringe into an aqueous sample in a conical centrifuge tube. A cloudy mixture containing fine droplets of the extraction solvent dispersed entirely in the aqueous phase is formed. The organic phase drop, which precipitates in the bottom of the tube after centrifugation, is injected into an injection port of gas chromatography (GC) or high-performance liquid chromatography (HPLC) for further analysis.

Lately, a novel microextraction technique called ultrasound-assisted emulsification microextraction (USAEME) was developed by Garcia-Jares and co-workers [23]. In USAEME, a very small volume of water-immiscible extraction solvent is mixed with an aqueous sample solution by ultrasound-assisted emulsification to form fine droplets for extracting analytes, obviating the need of a dispersive solvent in DLLME [24]. The ultrasound-assisted emulsification is carried out at 25°C for 10 min [23, 25] or for 9 min [26]. Recently, a few reports indicated that the use of manual shaking before ultrasound-assisted emulsification enhanced extraction efficiency. Fuh and co-workers used ultrasound with occasional manual shaking to generate a cloudy suspension [26]. The approach reported by Fontana and co-workers mixed honey samples with extraction solvent, Triton X-114, and used manual shaking to generate a homogeneous solution [27]. The work performed by Huang et al. showed that manual shaking for 10 s before ultrasound-assisted emulsification enhances the extraction efficiency of organochlorine pesticides (OCPs) by >100% in aqueous samples [28].

In this study, a new technique, manual shaking-enhanced, ultrasound-assisted emulsification microextraction (MS-USAEME) has been developed. Carbamate pesticides were chosen as the target analytes to evaluate the performance of the proposed method. Carbamate pesticides have been used for decades in many countries to increase agricultural production, are acetylcholinesterase inhibitors that allow acetylcholine to accumulate in the human body, resulting in health problems. Their residues can appear in fruit and vegetables, and are usually distributed in aqueous environments by leaching and runoff from soil into ground and surface water because of their high solubilities in water [29-31]. In order to detect trace amounts of the pesticides, the effects of changes of various experimental parameters, such as the nature and volume of the extraction solvent, duration of ultrasound emulsification, the effect of manual shaking and the addition of salt, were investigated and optimized. The MS-USAEME technique is simple and efficient. The objective of this study was to investigate the use of MS-USAEME for the extraction of carbamate pesticides from field water and vegetable matter.

2. Experimental

2.1. Reagents and materials

The carbamates, propoxur (99.8% purity) and pirimicarb (99% purity), were purchased from Fluka (Steinheim, Germany). Carbaryl, (99.8% purity) and methiocarb (99.5% purity) were purchased from Chem Service (West Chester, PA, USA). Carbofuran (98% purity) was purchased from Aldrich (Saint Louis, MO, USA). Stock 1 g L⁻¹ solutions of each pesticide were prepared in methanol (HPLC-grade) and stored at 4 ºC. Mixed working standard solutions
were prepared daily with deionized (DI) water purified with a Milli-Q system (Millipore, Bedford, MA, USA).

1-Octanol (99% purity), 1-nonanol (98% purity), 1-decanol (98% purity) and sodium chloride (NaCl, 99.5% purity) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). 1-Undecanol was purchased from TCI (Tokyo, Japan). HPLC-grade methanol was purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA).

2.2. Sample preparation

Field water samples (from Cyonglin, Hsinchu, Taiwan) were passed through a 0.45 µm pore size membrane filter (Millipore, Bedford, MA, USA) and stored at 4 ºC.

A sample of lettuce (Lactuca sativa) (from Siluo Township, Yunlin County, Taiwan) was chopped in a food chopper, then 2 L of deionized (DI) water was added per gram of chopped lettuce and the mixture homogenized in a food homogenizer. A 15 mL sample of the homogenized lettuce was centrifuged at 1398 × g for 10 min in a benchtop centrifuge; the supernatant liquid was then passed through a 0.45 µm pore size membrane (Millipore, Bedford, MA, USA) and stored at 4 ºC.

2.3. Instrumentation

Analysis was performed on an Agilent gas chromatograph (6850 series, Wilmington, DE, USA) equipped with a split/splitless injector and coupled with an Agilent mass spectrometer (5978B series). A DB-5 MS UI fused silica capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness; J & W Scientific, Folsom, CA, USA) was used for separation of the analytes. Initially the column temperature was held at 140 ºC for 1 min, then ramped to 270 ºC at 20 ºC min⁻¹ and kept at that temperature for 3.5 min. The carrier gas was helium (purity 99.9995%) that had been further purified by passage through an Agilent helium gas purifier (model RMSH-2) and the flow rate was 1.0 mL min⁻¹. The inlet was operated at 300 ºC and was used in the pulsed splitless mode. Ionization was operated in the electron impact (EI) mode at 70 eV. The temperature of the ion source was 230 ºC and the temperature of the quadrupole mass filter was 150 ºC. The MS was operated in full scan mode and a mass range of m/z 50–250 was scanned to confirm the retention times of the analytes. The selected ion monitoring (SIM) mode was used for the determination of the target compounds. Two selection ions were used for quantitation, and scan start times of the compounds were studied by gas chromatography-mass spectrometry (GC-MS): m/z 110, 152 (Propoxur); m/z 103, 164 (Carbofuran); m/z 115, 144 (Carbaryl); m/z 109, 153 (Methiocarb); m/z 166, 238 (Pirimicarb). The mass spectrometer was turned ON at 3.5 min and OFF after 11 min, to avoid filament breaking.

The ultrasonic water bath was obtained from Branson Ultrasonics (Danbury, CT, USA). The ultrasound frequency and power were 42 Hz and 100 W, respectively.

2.4. Analytical procedure

A 10 mL sample of DI water was placed in a glass centrifuge tube and spiked with five carbamate analytes at the required concentrations; 2.5 g of NaCl was added and dissolved
completely. A 10 µL portion of 1-octanol (the extraction solvent) was added and the tube was manually shaken gently for 10 s, and then immersed in an ultrasonic water bath. The levels of water bath and solution in the tube were both the same. During ultrasonication for 3 min at room temperature, the bath and the tube contents became cloudy due to the dispersion of fine droplets of 1-octanol within the aqueous bulk. After centrifugation at 1398 × g for 3 min in a benchtop centrifuge, the extraction solvent floated on the aqueous phase; the floating extraction phase (3 µL) was collected with a 25 µL microsyringe and transferred to a microtube. One µL of this extractant was injected into the GC for analysis (Figure 1). The above procedure was applied to field water and to the supernatant liquid from the lettuce samples mentioned in section 2.2.

Figure 1. The analytical procedure.

3. Results and discussion

3.1. Optimization of the experimental conditions

In order to obtain the most effective extraction of carbamate pesticides, it is important to determine the optimum conditions for the analysis. The variable parameters include the nature and volume of the extraction solvent, the ultrasonication time, the ionic strength and the effect of manual shaking. The behavior of five carbamate pesticides was studied under various extraction conditions. The calculation of enrichment factor (EF) for this method was (concentration of analyte in the floating phase, $C_{org}$) divided by (initial concentration of analyte in the aqueous sample, $C_0$).

$$\text{EF} = \frac{C_{org}}{C_0}$$
3.1.1. Selection of extraction solvent

The selection of an extraction solvent is the most important experimental parameter in this method. An appropriate extraction solvent must have: (1) low toxicity, (2) immiscibility with water and (3) high extraction ability for the target analytes. On the basis of these criteria, to achieve good extraction of carbamates from aqueous samples, alcohols 1-octanol, 1-nonanol, 1-decanol and 1-undecanol were chosen as potential extraction solvents. The final selection of solvent was decided on the basis of extraction efficiency. Comparison of EF obtained with each of the four extraction solvents showed that 1-octanol was the most effective; see Figure 2. It seems that carbamates have a better affinity for a slightly polar solvent, 1-octanol.

![Figure 2. Selection of extraction solvent (n= 3). Samples were spiked with 50 μg L⁻¹ of each analyte. Extraction conditions: aqueous sample volume 10.0 mL; extraction solvent volume 15 μL; ultrasonication time: 5 min; salt addition: 1 g NaCl.](http://dx.doi.org/10.5772/55971)

3.1.2. Effect of the volume of extraction solvent

The volume of the floating phase is increased with increasing volume of the extraction solvent; however, the analytes are diluted as a result of the increased volume of the floating phase. To estimate the influence of volume of extraction solvent on the procedure, the volume of extraction solvent (1-octanol) was varied in the range 8 to 15 µL. The results showed that the EF of the analytes decreased when the volume of the extraction solvent was added above 10 µL. This may be due to the dilution of extracts (Figure 3). Therefore, 10 µL of extraction solvent was chosen as the optimal volume for further studies.
3.1.3. Effect of ultrasonication time

The ultrasonication time might affect extraction efficiency because it affects both emulsification and the mass transfer process. To examine this effect, the EF was monitored with extraction times varying from 0 to 7 min (Figure 4). The maximum EF was achieved after ultrasonication for 3 min and no significant variation was observed with extraction times longer than 3 min. This is probably due to the fact that the ultrasonic water bath generates the emulsion quickly, rapidly making a very large contact surface area between the extraction solvent and the aqueous phase. Therefore 3 min was taken to be the optimum extraction time.

3.1.4. Effect of manual shaking

Manual shaking was essential to ensure that the extraction solvent and aqueous samples were adequately mixed before the ultrasound treatment when the ultrasonic extraction time was as brief as 3 min. A study done by Huang and co-workers [28] found that maximum peak area was achieved with 10 s of manual shaking and there was no significant increase for longer times. Here, with manual shaking, the EF was significantly higher than the EF value obtained from no manual shaking. The shaking assured that the aqueous sample was mixed well with the extraction solvent, thus generating the emulsion quickly (Figure 5).
Figure 4. Effect of ultrasonic extraction time (n = 3). Samples were spiked to 50 μg L\(^{-1}\) of each analyte. Extraction conditions: aqueous sample volume 10.0 mL; extraction solvent 1-octanol; extraction solvent volume 10 μL; salt addition: 1 g NaCl.

Figure 5. Effect of hand-shaking (n = 3). Samples were spiked to 50 μg L\(^{-1}\) of each analyte. Extraction conditions: aqueous sample volume 10.0 mL; extraction solvent 1-octanol; extraction solvent volume 10 μL; ultrasonication time: 3 min; salt addition: 1 g NaCl.
3.1.5. Effect of the amount of salt added

Ionic strength is an important determinant of extraction efficiency. During the extraction procedure, adding salt increases ionic strength, which leads to the “salting out” phenomenon, commonly discussed in liquid-liquid extraction. The presence of salt decreases the solubility of target analytes in aqueous phases, and improves their partition from aqueous to organic layer. This was observed on the increasing EFs when NaCl was added from 0 to 2.5 g (Figure 6). However, adding salt also eliminates the emulsion between aqueous and organic layers which results in the larger volume of floating phase left after extraction. When more salt was added, more floating phase was obtained. The larger volume of floating phase offsets the enhanced partition of the pesticides in organic layer. There was no significant change of the EF as the amount of salt added was increased from 2.5 to 3.0 g. On the basis of this result, 2.5 g of NaCl was added to the aqueous sample solution for further studies.

![Graph showing the effect of salt addition on EFs](image)

Figure 6. Effect of salt addition (n = 3). Samples were spiked to 50 μg L\(^{-1}\) of each analyte. Extraction conditions: aqueous sample volume 10.0 mL; extraction solvent 1-octanol; extraction solvent volume 10 μL; ultrasonication time: 3 min.

3.2. Comparison of methods

The proposed method requires simple equipment: a 25 μL syringe, 10 μL of low toxicity extraction solvent, and an ultrasonic water bath, used to emulsify the extraction solvent and sample to form fine droplets for extraction. Compared to DLLME, solvent terminated dispersive liquid-liquid microextraction (ST-DLLME) and dispersive liquid-liquid microextraction
combined with sweeping micellar electrokinetic chromatography (DLLME-sweeping-MEKC) method, this method avoids the use of a high toxicity extraction solvent and a large amount of dispersive solvent, which increases the solubility of the analytes in water during extraction. Unlike other extraction methods such as SPE, SPME, LPME, single drop microextraction (SDME) and quick, easy, cheap, effective, rugged, and safe (QuEChERS)-based extraction, there is no need for this method to use a packed solid phase cartridge, a fragile fiber coated with a polymeric phase, a section of hollow fiber, a metal stand or clean-up sorbent. The apparatus needed for MS-USAEME is simpler than that required by the above-mentioned methods. According to the results in Table 1, the proposed method, which uses less organic solvent, maintains the advantage of short extraction times and makes the extraction more efficient.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Linearity (ng mL(^{-1}))</th>
<th>MDL (ng mL(^{-1}))</th>
<th>RSD (%)</th>
<th>Extraction time (min)</th>
<th>EF</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF-LPME–HPLC-UV</td>
<td>1–1,000</td>
<td>0.024–5.5</td>
<td>1.90–9.53</td>
<td>30</td>
<td>294-873</td>
<td>[32]</td>
</tr>
<tr>
<td>HF-LPME–GC–MS</td>
<td>1–400</td>
<td>0.2–0.8</td>
<td>4.86–7.81</td>
<td>20</td>
<td>37-144</td>
<td>[30]</td>
</tr>
<tr>
<td>SPME–GC–MS</td>
<td>–</td>
<td>1.2–4.6</td>
<td>13–17</td>
<td>120</td>
<td>-</td>
<td>[33]</td>
</tr>
<tr>
<td>SPME–HPLC-MS</td>
<td>50–5,000</td>
<td>1–10</td>
<td>1–6</td>
<td>90</td>
<td>-</td>
<td>[34]</td>
</tr>
<tr>
<td>DLLME–HPLC-UV</td>
<td>5–500</td>
<td>0.4–1.0</td>
<td>4.7–6.5</td>
<td>1</td>
<td>101-145</td>
<td>[35]</td>
</tr>
<tr>
<td>ST-DLLME–GC–MS</td>
<td>0.005–20</td>
<td>0.001–0.5</td>
<td>2.3–6.8</td>
<td>10</td>
<td>-</td>
<td>[36]</td>
</tr>
<tr>
<td>UASEME–HPLC-DAD</td>
<td>0.3–200</td>
<td>0.1–0.3</td>
<td>3.4–4.8</td>
<td>3</td>
<td>170-246</td>
<td>[37]</td>
</tr>
<tr>
<td>DLLME-sweeping-MEKC(^{c})</td>
<td>10–500</td>
<td>2.0–3.0</td>
<td>4.7–6.5</td>
<td>1</td>
<td>491-1834</td>
<td>[38]</td>
</tr>
<tr>
<td>SDME–GC–MS</td>
<td>0.05–200</td>
<td>0.02</td>
<td>0.6–13.1</td>
<td>15</td>
<td>-</td>
<td>[39]</td>
</tr>
<tr>
<td>QuEChERS–LC-MSMS</td>
<td>1–20</td>
<td>1</td>
<td>&lt;20</td>
<td>21</td>
<td>-</td>
<td>[40]</td>
</tr>
<tr>
<td>This work</td>
<td>0.05–100</td>
<td>0.013–0.026</td>
<td>6.8–16.9</td>
<td>3</td>
<td>237-638</td>
<td>This work</td>
</tr>
</tbody>
</table>

a) HF-LPME: hollow fiber liquid phase microextraction.
b) SPME: solid phase microextraction.
c) DLLME: dispersive liquid microextraction.
d) ST-DLLME: solvent terminated dispersive liquid-liquid microextraction
e) DLLME-sweeping-MEKC: dispersive liquid-liquid microextraction combined with sweeping micellar electrokinetic chromatography
f) SDME: single drop microextraction.
g) QuEChERS: quick, easy, cheap, effective, rugged and safe procedure.

Table 1. Comparison of methods.
3.3. Analytical performance

Linearity (LR), regression coefficient ($R^2$) and EF were investigated under optimized experimental conditions. The LR of the method was evaluated using water samples spiked with the selected compounds at various concentrations. The performance of the proposed method is summarized in Table 2. The linear calibration of the targeted carbamate pesticides was examined in the range 0.05 to 100 µg L$^{-1}$. Linear plots yielded $R^2 \geq 0.9972$. The EF for all of the carbamate pesticides tested was in the range from 237 to 638. The results indicate that MS-USAEME combined with GC-MS was sensitive enough for the detection of these five carbamate pesticides.

<table>
<thead>
<tr>
<th>Carbamate</th>
<th>LR (µg L$^{-1}$)</th>
<th>$R^2$</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propoxur</td>
<td>0.05–100</td>
<td>0.9987</td>
<td>444</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>0.05–100</td>
<td>0.9988</td>
<td>638</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>0.05–100</td>
<td>0.9993</td>
<td>365</td>
</tr>
<tr>
<td>Methiocarb</td>
<td>0.05–100</td>
<td>0.9972</td>
<td>266</td>
</tr>
<tr>
<td>Pirimicarb</td>
<td>0.05–100</td>
<td>0.9980</td>
<td>237</td>
</tr>
</tbody>
</table>

Propoxur : 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 20, 100 µg L$^{-1}$
Carbofuran : 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 20, 100 µg L$^{-1}$
Carbaryl : 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 20, 100 µg L$^{-1}$
Methiocarb : 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 20, 100 µg L$^{-1}$
Pirimicarb : 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 20, 100 µg L$^{-1}$

Table 2. Analytical performance.

3.4. Analysis of real samples

In order to investigate the influence of the sample matrix in real samples, the determination of carbamate pesticides in water and vegetable matter was done using the method described here (Figure 7). All of the real samples were spiked with 5 µg L$^{-1}$ methiocarb and 0.1 µg L$^{-1}$ of the other four analytes to calculate the recovery of the targeted compounds. The reproducibility of the method was satisfactory; the RSD ranged from 6.8 to 16.9%. The relative recoveries were calculated by the ratios of the concentration of the carbamate pesticides in real samples and the concentrations of the analytes extracted in ultrapure water samples. Both samples were spiked with the same amount of pesticides. For all target compounds, the relative recoveries of field water and vegetables were within 77 to 114%. The definition of absolute recovery was determined by the ratio of extracted concentration in real samples and concentration spiked in the real sample. The absolute recoveries of target analytes were between 5.3 to 30.6%. The method detection limit (MDL) was calculated as three times the standard deviation of seven replicate runs of water and vegetable samples spiked with low concentrations of the analytes. MDL ranged from 0.013 to 0.022 µg L$^{-1}$ for field water and 0.017 to 0.026 µg L$^{-1}$ for the vegetable sample (Table 3).
Figure 7. GC-MS chromatogram of A) field water sample and B) lettuce spiked with 5 carbamate pesticides at 0.5 µg L\(^{-1}\). Extraction conditions: samples volume 10.0 mL; extraction solvent 1-octanol; extraction solvent volume 10 μL; ultrasonication time: 3 min; salt addition: 2.5 g NaCl. 1. Propoxur. 2. Carbofuran. 3. Carbaryl. 4. Methiocarb. 5. Pirimicarb.

Table 3. Analysis of real samples.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Field water</th>
<th></th>
<th></th>
<th>Vegetable sample</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDL (µg L(^{-1}))</td>
<td>RSD% (n=6)</td>
<td>Absolute recovery (%)(^{a})</td>
<td>Relative recovery (%)(^{b})</td>
<td>MDL (µg L(^{-1}))</td>
<td>RSD% (n=6)</td>
</tr>
<tr>
<td>Propoxur</td>
<td>0.017</td>
<td>8.2</td>
<td>17.3</td>
<td>82</td>
<td>0.022</td>
<td>7.9</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>0.022</td>
<td>10.9</td>
<td>30.6</td>
<td>114</td>
<td>0.026</td>
<td>7.2</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>0.019</td>
<td>6.8</td>
<td>13.1</td>
<td>77</td>
<td>0.023</td>
<td>11.3</td>
</tr>
<tr>
<td>Methiocarb</td>
<td>0.017</td>
<td>16.9</td>
<td>7.9</td>
<td>110</td>
<td>0.017</td>
<td>8.0</td>
</tr>
<tr>
<td>Pirimicarb</td>
<td>0.013</td>
<td>8.2</td>
<td>10.3</td>
<td>85</td>
<td>0.023</td>
<td>11.5</td>
</tr>
</tbody>
</table>

\(^{a}\) Absolute recoveries were determined by the ratio of the extracted concentration to the spiked concentration in the real sample.

\(^{b}\) Relative recoveries were determined by the ratio of the concentration found in the real sample to the concentration in deionized water samples. Both samples were spiked with the same amount of analytes. The extraction yields obtained from deionized water were considered as 100%.

Concentrations used for testing absolute recovery and relative recovery were: propoxur: 0.1 µg L\(^{-1}\); carbofuran: 0.1 µg L\(^{-1}\); carbaryl: 0.1 µg L\(^{-1}\); methiocarb: 5 µg L\(^{-1}\); pirimicarb: 0.1 µg L\(^{-1}\). MDL: spiked with 0.05 µg L\(^{-1}\) each compound.
4. Concluding remarks

This paper describes a simple, rapid extraction method using GC-MS to analyze carbamate pesticides in field aqueous and lettuce samples. Manual shaking for 10 s before ultrasonication is essential for effective extraction when the ultrasonication time is as brief as 3 min. The method performed well; repeatability, EF and recovery were satisfactory and the analysis can be done in a short time. Compared to other microextraction methods, the method described here is environmentally friendly and has the advantages of speed, simplicity, frugal use of organic solvent (10 µL/sample) and low cost.

Acknowledgements

This study was supported by the National Science Council of Taiwan (NSC 99-2113-M-007-004-MY3).

Author details

Pin-Huei Chen¹, Pai-Shan Chen²* and Shang-Da Huang¹

*Address all correspondence to: sduang@mx.nthu.edu.tw; paishanchen@ntu.edu.tw

1 Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan

2 Department and Graduate Institute of Forensic Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan

References


