Talanta 86 (2011) 142–147

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Automatic heating and cooling system in a gas purge microsyringe extraction

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ARTICLE INFO

Article history:
Received 10 April 2011
Received in revised form 19 August 2011
Accepted 22 August 2011
Available online 6 September 2011

Keywords:
Microextraction
Heating
Cooling
Gas purge
GC–MS

ABSTRACT

The gas purge microsyringe extraction (GP-MSE) technique offers quantitative and simultaneous extraction, and rapid gas chromatographic–mass spectrometric determination of volatile and semivolatile chemicals is possible. To simplify the application, a new automatic temperature control system was developed here. Stable heating and cooling over a wide range of temperatures were achieved using a micro-heater and thermoelectric cooler under varying gas flow conditions. Temperatures could be accurately controlled in the range 20–350 °C (heating) and 20 to −4 °C (cooling). Temperature effects on the extraction performance of the GP-MSE were experimentally investigated by comparing the recoveries of polycyclic aromatic hydrocarbons (PAHs) under various experimental conditions. A sample treatment was completed within 3 min, which is much less than the time required for chromatographic analysis. The recovery of chemicals determined ranged from 81 to 96%. High reproducibility data (RSD ≤ 5%) were obtained for direct extraction of various analytes in spiked complex plant and biological samples. The data show that the heating and cooling system has potential applications in GP-MSE system for the direct determination of various kinds of volatile and semivolatile chemicals from complex matrices without any, or only minor, sample pretreatment.

1. Introduction

Rapid modern instrumental techniques often require a disproportionate amount of time for sample pre-treatment. In some instances, sample preparations take up to 80% of the total time of a complete separation-based analytical process [1]. For this reason many studies related to chromatographic analysis have focused on simple, fast and automatic extraction [2–8] and clean up [9–12] of complex samples before determination by GC–MS or LC/MS.

For quantitative GC–MS determination of volatile and semivolatile chemicals, a gas purge microsyringe extraction (GP-MSE) technique was recently introduced by Yang and coworkers [13]. This approach integrates extraction, cleanup and concentration procedures. In this method, heating and cooling temperature are important factors affecting extraction efficiency and accuracy. The analytes in the matrix are released to the headspace at high temperature and are enriched in a micro volume of solvent which is kept in the liquid phase at low temperature by a cooling system.

Up to now, many kinds of heating techniques, such as steam distillation in the headspace liquid phase microextraction (HS-LPME) [14], microwave heating in the headspace single drop microextraction (HS-SDME) [15], and recycled hot water in the headspace solvent microextraction [16] have been widely studied. Cooling temperature also affects extraction efficiency because the extraction process is exothermic. The capacity of the organic solvent and the partition coefficient (K_{oh}) of analytes between gas phase and extracting solvent increase with decreasing temperature of the extraction solvent [4,16–19]; this is more pronounced in microextraction techniques due to limitation of extractant volume. To achieve low temperatures and high capacity of the microextraction phase, a recycled cold water system [16,19], CO_2 cooling [20,21], and thermoelectric cooling [22] have been applied during the last few decades.

To achieve simple automatic heating and cooling in the GP-MSE system, a metal-oxide ceramic heater (MCH) and thermoelectric cooler (TEC) were fabricated and evaluated experimentally here. A xylene, polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and alkylphenols (APs) which are covering wide range of boiling temperature were used as target compounds. The results show that the system has several advantages such as simple fabrication, high heating and cooling efficiency and automation, miniaturization, and finally is suitable for wide application in microextraction techniques.

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0039-9140/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.talanta.2011.08.047

2. Experimental

2.1. Material and solvent

The materials and components for construction of this apparatus were obtained from the following suppliers: the metal-oxide ceramic heater (MCH) from Tiancheng Thermistors (China); the platinum resistor sensor from Hayashi Denko (Japan); a semiconductor refrigeration component from Fijitaka (Japan); the heat sink, Shenzhen Walmate Electronics (China); the cooling fan from Fengshengyuan Electronics (Shenzhen, China); Perspex from JAGI (Shanghai, China); the gas mass flow controller (S49-32B/MT) from Beijing Metron Instruments (China); the AT89C52 microcontroller, Atmel (USA); the operational amplifier (LM124) and 12-bit analog-to-digital converter (ADS1286) from Texas Instruments (USA); the 8-bit A/D and D/A converter (PCF8591) from Philips (Holland); the LCD module (LCM141) from Beijing Qingyun Hi-Tech Development (China).

Xylene, PAHs, OCPs, PCBs and APs standards, internal standards tetrachloro-*m*-xylene (TCMX), $^2H_{10}$-acenaphthene, $^2H_{12}$-perylene, and [$^2H_{10}$]-phenanthrene were purchased from Supelco (Bellefonte, PA, USA), and the purity of standards are higher than 99%. Organic solvents (hexane, dichloromethane, methanol and acetone) were HPLC grade obtained from Caledon (Georgetown, Ont., Canada). 20 mg L^{-1} of xylene, 0.4 mg L^{-1} of OCPs and 0.4 mg L^{-1} of PCBs were prepared in hexane, 20 mg L^{-1} of PAHs and 340 mg L^{-1} of APs were prepared in methanol and acetone, respectively. The standard solutions were stored at 0–4 °C.

2.2. Evaporation and extraction

The heating performance of the GP-MSE was evaluated using the evaporation efficiency (EvE) of the analytes selected. According to the mechanism of GP-MSE, the analytes adsorbed on the surface of the sample matrix should be vaporized into the gas phase (i.e., released to the headspace phase from the sample matrix) and then transported to the inner microsyringe barrel by gas purging, and then trapped by the organic solvent. EvE values were obtained with Eq. (1), where A_I is initial amount of analytes in the sample vial, A_{EP} is evaporated amount of analytes from the sample vial, and A_R is residual amounts of analytes in the sample vial after evaporation. The residual amount of chemicals in the sample vial was determined directly by GC–MS after addition of 200 μL of dichloromethane into the cooled sample vial.

$$\mathrm{EvE} = \frac{A_{EP}}{A_I} \times 100\% = \frac{(A_I - A_R)}{A_I} \times 100\% \quad (1)$$

In the GP-MSE technique, cooling of the extraction organic solvent has following advantages. One is to increase extraction efficiency; another is to reduce the amount of toxic organic solvent used. According to the extraction mechanism of the GP-MSE, the evaporated gas phase analytes are trapped by the liquid phase organic solvent after the gas purge, so the extraction efficiency (ExE) was calculated using following Eq. (2). Here, A_S is total amount of analyte in the microextraction solvent, and the other symbols have the same meaning as in Eq. (1).

$$\mathrm{E} \times \mathrm{E} = \frac{A_S}{A_{EP}} \times 100\% = \frac{A_S}{(A_I - A_R)} \times 100\% \quad (2)$$

2.3. Fabrication of the micro-heater and thermoelectric cooler

Unlike the apparatus previously introduced by Yang et al, a metal-oxide ceramic heater (MCH) which generates heat when an electric current is applied was used here to achieve high temperatures and rapid heating. The heater band was constructed using ceramic alumina. The MCH was fixed to the bottom of the ceramic alumina by mechanical compressive force. A cylindrical sample cell was machined in the middle of the micro-heater for the sample vial. To fit the experimental sample container, the depth of the sample cell is 10 mm and its diameter is 8 mm. The platinum resistor sensor was placed inside the ceramic alumina. The sensor measures the temperature of the sample cell and the signal is then sent to the automatic control system (see Section 2.4). A stainless steel gas pipe was connected to the bottom of the micro-heater the cylindrical hole inside the cutting sleeve and inserted into the micro-heater (Fig. 1).

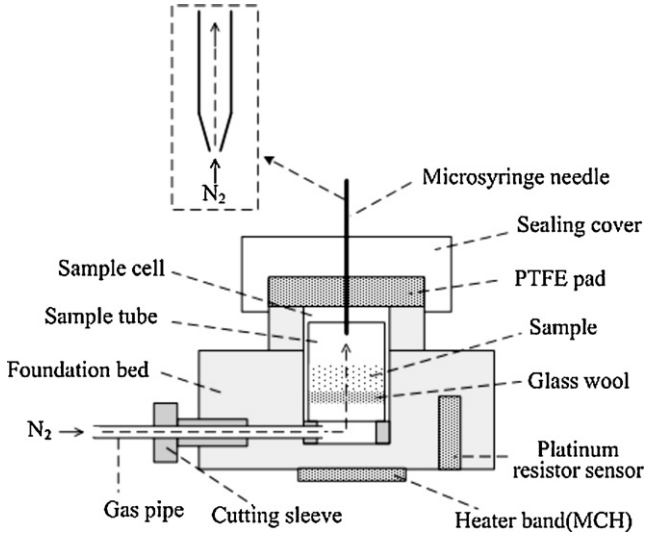

Fig. 1. Schematic diagram of the micro-heater component of the automated GP-MSE apparatus.

A simple and rapid thermoelectric cooler (TEC) was used in the solid phase microextraction (SPME) technique by Pawliszyn et al. [22]. The device provided relatively rapid, precise and sensitive extraction in the analysis of volatile compounds. The TEC system was applied in GP-MSE after slight modification of the device (a cold fiber SPME component) for cooling the microsyringe body. In addition, an automatic control system was added to the cooling device. As shown in Figs. 2 and 3, the TEC is composed of refrigeration piece, a platinum resistor sensor, an insulation cover, an aluminum box, an aluminum plate, and a heat sink and cooling fan. The heat sink and fan combination was attached on the hot side of the refrigeration piece through the aluminum plate. The cold side of the refrigeration piece was attached to the aluminum box. To place and cool the microsyringe, a hemi-cylindrical groove (diameter 8 mm) was machined in the aluminum box. The side of gap in the groove was exposed to the surface of the insulation cover, so that the behavior of the organic solvent and gas bubble could be observed from the gap (Fig. 3). The cover was made of Perspex, the whole body of the aluminum box was covered by the cover. A hole was made in the side of the aluminum box and insulation cover to embed the platinum resistor sensor used to detect the temperature of the microsyringe.

Overall, the heating and cooling components were connected with the microsyringe needle (Fig. 3). The analytes absorbed on the surface of the sample matrix were evaporated to the headspace of the sample vial by heating, and transferred to the microsyringe body through the microsyringe needle by the inert gas (nitrogen gas) purge; they were then extracted or trapped by the organic solvent in the microsyringe which was cooled by the TEC.

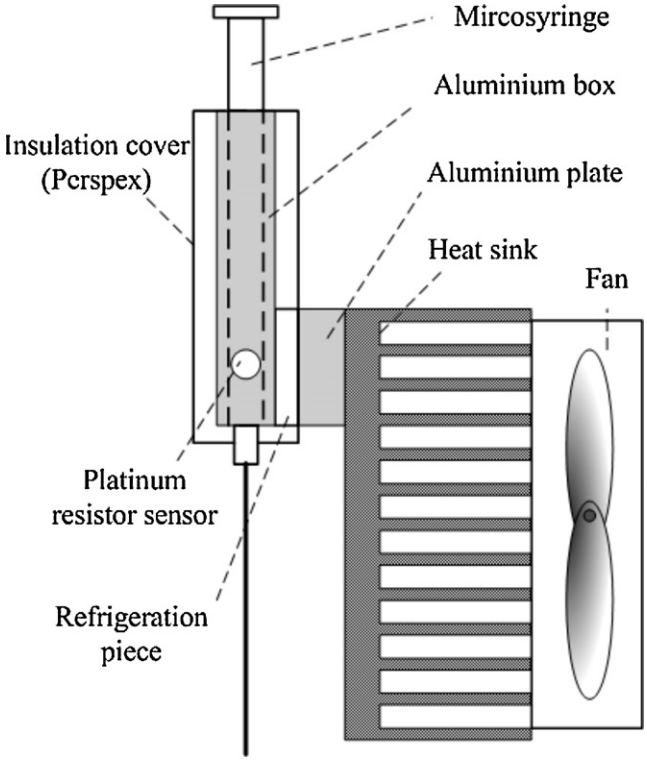

Fig. 2. Schematic diagram of the thermoelectric cooler component of the automated GP-MSE apparatus.

2.4. Automatic system

A switchable power supply is used; the keyboard is used to set the temperatures of the cooler and heater, the gas flow rate and extraction time. The LCD is designed to display these values. Electrical automatic system was consists of the microcontroller [23], a platinum resistor sensor, the operational amplifier, the 12-bit analog-to-digital converter [24], the drive circuit for the heater band, the A/D and D/A converter [25], the mass flow controller [26] and the LCD module [27]. To decrease errors in the analog temperature signal collected by the platinum resistor sensor, two A/D converters of 12-bit resolution are incorporated into the system. After setting the working parameters via the keyboard, the whole system should operate automatically.

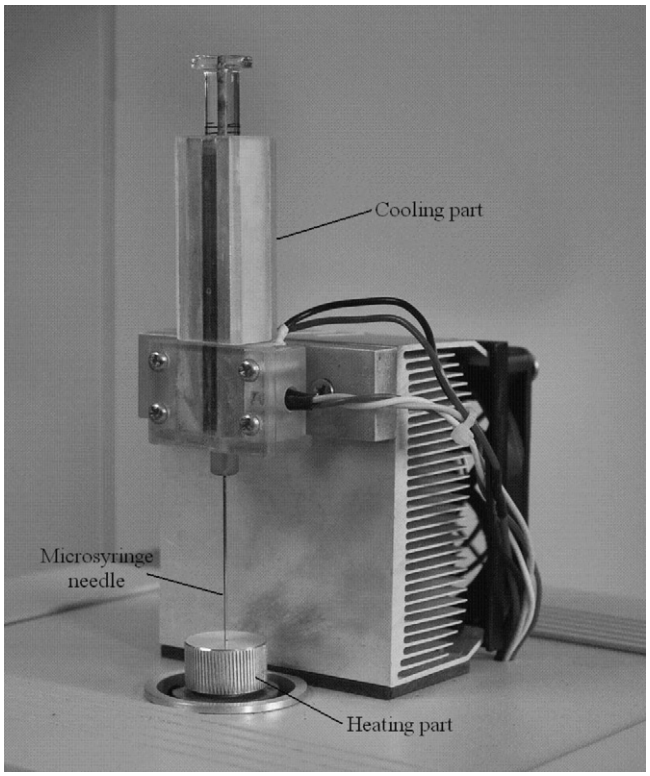

Fig. 3. Photograph of the thermoelectric cooling unit with heat sink.

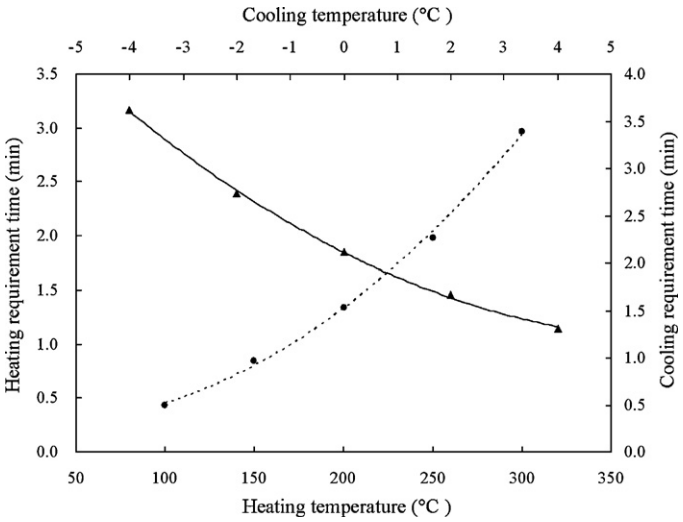

Fig. 4. The temperature behaviors of the micro-heater and thermoelectric cooler. The dotted line is heater and the solid line is cooler.

2.5. Experimental procedure

As mentioned in Section 2.2, heating and cooling have significant effects on the evaporation and extraction efficiency of target compounds. The performances of the heater and cooler were evaluated as follows. (1) A standard sample or real sample was put in a glass tube (9 mm × 7 mm). (2) The sample tube was put into the heating sample cell. (3) The microsyringe was inserted into the sample cell through the TEC and socket. (4) To start the extraction, a suitable extracting solvent was added to extraction microsyringe barrel after removal of the microsyringe plunger, while applying heating and cooling power and opening the gas valve. (5) After a defined period, the microsyringe is removed from the apparatus, and the 2 μL of extracting solvent is directly (or after dilution) injected into the GC–MS for analysis.

2.6. GC–MS analysis

Separation and detection of xylene, PAHs and APs were carried out using a Shimadzu GC 2010 equipped with quadruple mass spectrometer system (MS 2010) and a DB5 (5% biphenyl + 95% methylpolysiloxane) fused-silica capillary column with a 30 m × 0.25 mm i.d. and thickness 0.25 μm. Conditions for GC–MS analysis are described in reports by Yang et al. and Wang et al. [9], respectively. Quantitative analysis of OCPs and PCBs were carried out using a Shimadzu GC 2010 with an electron capture detector (ECD); other GC conditions were similar to those reported by Hong et al. [28]. To reduce the effects of instrumental analysis conditions, the internal standard method was used for quantification of analytes.

Table 1
Residual amounts of extraction solvent in the microsyringe barrel under different heating, cooling and gas flow rates in the GP-MSE technique.

Gas flow rate (mL min^{-1})	Dichloromethane (μL)				Hexane (μL)				Acetone (μL)			
	150 °C		300 °C		150 °C		300 °C		150 °C		300 °C	
	0 °C	−4 °C	0 °C	−4 °C	0 °C	−4 °C	0 °C	−4 °C	0 °C	−4 °C	0 °C	−4 °C
1	47.5	47.5	47.5	47.5	50	50	50	50	50	50	50	50
3	35	40	35	40	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5
5	20	27.5	20	27.5	35	37.5	35	37.5	35	37.5	35	37.5

3. Results and discussion

3.1. Heating and cooling rate

Initial temperatures of the heater and cooler were controlled at 20 °C (room temperature), and the final set temperatures were measured using the platinum resistor sensor. The analysis time was measured by stopwatch. For applications to the determination of volatile and semivolatile chemicals from real samples matrix, the final heating temperatures were set at 100, 150, 200, 250 and 300 °C, and the final cooling temperatures were set at 4, 2, 0, −2 and −4 °C, and the gas flow rate was controlled at 2 mL min^{-1}. Observed temperatures were plotted against records heating and cooling times. As shown in Fig. 4, only 3.0 min are required for heating the sample cell from room temperature to 300 °C, and 3.6 min are required for cooling from room temperature to −4 °C.

3.2. Stability of the temperature

The gas flow rate is an essential factor in the GP-MSE technique, while it may affect the thermal stability of the heating and cooling systems. To understand the effect of gas flow on temperature stability, heating and cooling set temperatures were measured continuously over 20 min working time under various gas flow conditions. Heating temperatures were set at 100, 200 and 300 °C, cooling temperatures at, 0 and −4 °C, and gas flow rates set at 1, 3, and 5 mL min^{-1}. The experimental data demonstrate that both heating and cooling set temperatures were essentially constant under any condition of gas flow. The stability of heating and cooling temperatures favors high precision of analyte recovery in the GP-MSE technique, which was discussed in the following sections.

3.3. Evaporation profile of target chemicals

Heating temperature affects the evaporation of the target chemicals, and finally it affects on extraction recovery. The evaporation profiles of analytes were studied by monitoring the evaporation efficiency of PAHs chosen to cover a wide range of boiling points. The gas flow rate was set to 2 mL min^{-1} and heating time was set to 2 min in these experiments. The sample amount was 40 ng for each of the PAHs determined. As shown in Fig. 5, the evaporation efficiency of higher weight PAHs significantly increased with increasing heater temperature, particularly for dibenz[*a*,*h*]anthracene and benzo[*ghi*]perylene. For example, 88% of benzo[*ghi*]perylene remained in the sample vial at the 150 °C, but residual benzo[*ghi*]perylene was hardly detectable (<5%) after treatment of the sample vial at 300 °C. This is indicating that analytes are easily released from the sample matrix into the headspace at higher sample temperature under these gas flow conditions. Based on these experimental results, sample vial temperature of 300 °C was chosen for the remainder of this study.

3.4. Extraction efficiency and usage of solvent

As mentioned above, evaporated chemicals are extracted by the micro amount of solvent in the GP-MSE technique. The temperature and the volume of the extraction solvent are therefore directly related to extraction efficiency.

The extraction efficiency data on the analytes selected were obtained at −4, 0 and 20 °C, and the results were compared. The gas flow rate of 2 mL min^{-1} and heater temperature of 300 °C was used, and sample weight was standardised at 40 ng xylene and PAHs. As can be seen in Fig. 6, the recovery of most target compounds

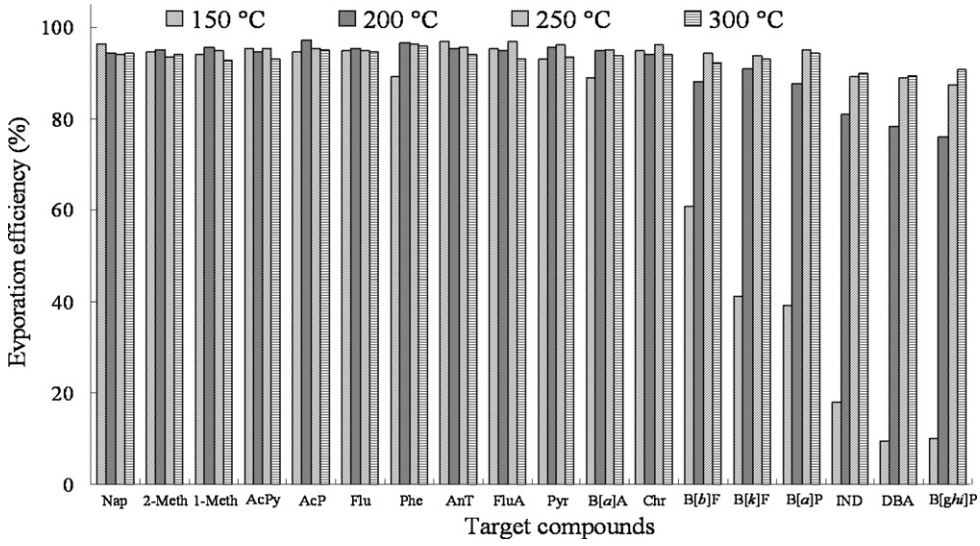

Fig. 5. Evaporation profiles of PAH analytes at the different sample temperature. Nap: naphthalene; 2-Meth: 2-methylnaphthalene; 1-Meth: 1-methylnaphthalene; AcPy: acenaphthylene; AcP: acenaphthene; Flu: fluorene; Phe: phenanthrene; AnT: anthracene; FluA: fluoranthene; Pyr: pyrene; B[*a*]A: benzo[*a*]anthracee; Chr: chrysene; B[*b*]F: benzo[*b*]fluoranthene; B[*k*]F: benzo[*k*]fluoranthene; B[*a*]P: benzo[*a*]pyrene; IND: indeno[1,2,3-*cd*]pyrene; DBA: dibenz[*a*,*h*]anthracene; B[*ghi*]P: benzo[*ghi*]perylene.

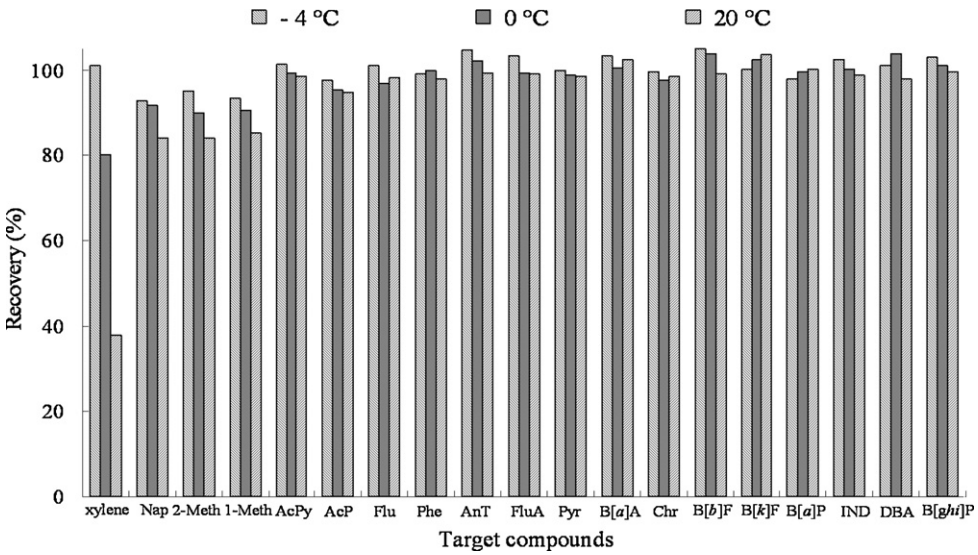

Fig. 6. Effect of cooling temperature on the extraction efficiency. Nap: naphthalene; 2-Meth: 2-methylnaphthalene; 1-Meth: 1-methylnaphthalene; AcPy: acenaphthylene; AcP: acenaphthene; Flu: fluorene; Phe: phenanthrene; AnT: anthracene; FluA: fluoranthene; Pyr: pyrene; B[*a*]A: benzo[*a*]anthracee; Chr: chrysene; B[*b*]F: benzo[*b*]fluoranthene; B[*k*]F: benzo[*k*]fluoranthene; B[*a*]P: benzo[*a*]pyrene; IND: indeno[1,2,3-*cd*]pyrene; DBA: dibenz[*a,h*]anthracene; B[*ghi*]P: benzo[*ghi*]perylene.

Table 2
Summary of extraction results for various chemicals in spiked real samples using GP-MSE and Soxhlet extraction (SE) techniques.

Compounds*	Concentration (ng g^{-1})					
	Spiked plant sample (n = 3)			Spiked carp sample (n = 3)		
	GP-MSE	SE	GP-MSE/SE	GP-MSE	SE	GP-MSE/SE
PAHs						
Nap	20.43	21.31	0.96	273.7	261.3	1.05
2-Meth	13.73	14.03	0.98	213.7	214.0	1.00
1-Meth	8.52	8.71	0.98	208.5	218.1	0.96
AcPy	6.02	5.87	1.03	253.4	245.9	1.03
AcP	5.81	5.11	1.14	260.1	257.1	1.01
Flu	11.13	10.61	1.05	290.0	310.6	0.93
Phe	33.65	34.19	0.98	327.3	334.2	0.98
AnT	5.93	5.53	1.07	227.1	225.5	1.01
FluA	18.53	17.97	1.03	246.3	217. 7	1.13
Pyr	18.25	19.01	0.96	325.4	319.0	1.02
B[*a*]A	7.73	6.94	1.11	219.4	216.4	1.01
Chr	12.58	12.05	1.04	263.1	232.5	1.13
B[*b*]F	11.72	12.17	0.96	229.1	212.7	1.08
B[*k*]F	4.25	4.43	0.96	215.1	224.3	0.96
B[*a*]P	5.91	6.02	0.98	168.3	206.0	0.82
IND	8.25	8.09	1.02	200.2	208.0	0.96
DBA	5.54	5.37	1.03	186.1	200.3	0.93
B[*ghi*]P	6.85	6.59	1.04	187.4	206.5	0.91
APs						
4-*t*-OP	5.84	5.32	1.10	205.48	215.2	0.95
4-*t*-NP	20.31	20.07	1.01	230.31	220.1	1.05
OCPs						
α-HCH	5.32	5.29	1.01	211.3	201.3	1.05
γ-HCH	5.07	5.10	0.99	232.0	222.4	1.04
p,p′-DDE	5.41	5.72	0.95	205.4	215.2	0.95
p,p′-DDD	5.57	5.42	1.03	201.5	191.8	1.05
p,p′-DDT	5.05	5.12	0.99	256.0	241.9	1.06
PCBs						
PCB 29	5.72	5.31	1.08	211.7	201.5	1.05
PCB 44	8.36	8.12	1.03	208.3	198.1	1.05
PCB 105	5.53	5.41	1.02	235.3	215.9	1.09
PCB 128	5.61	5.42	1.04	213.6	235.2	0.91
PCB 153	6.71	6.28	1.07	200.1	210.8	0.95

Abbreviations: Nap = naphthalene; 2-Meth = 2-methylnaphthalene; 1-Meth = 1-methylnaphthalene; AcPy = acenaphthylene; AcP = acenaphthene; Flu = fluorene; Phe = phenanthrene; AnT = anthracene; FluA = fluoranthene; Pyr = pyrene; B[*a*]A = benzo[*a*]anthracene; Chr = chrysene; B[*b*]F = benzo[*b*]fluoranthene; B[*k*]F = benzo[*k*]fluoranthene; B[*a*]P = benzo[*a*]pyrene; IND = indeno[1,2,3-*cd*]pyrene; DBA = dibenz[*a,h*]anthracene; B[*ghi*]P = benzo[*ghi*]perylene; 4-*t*-OP = 4-*t*-octylphenol; 4-*t*-NP = 4-*t*-nonylphenol; α-HCH = alpha-hexachlorocyclohexane; γ-HCH = gamma-hexachlorocyclohexane; *p,p′*-DDE = *p,p′*-dichlorodiphenyldichloroethylene; *p,p′*-DDD = *p,p′*-dichlorodiphenyldichloroethane; *p,p′*-DDT = *p,p′*-dichloro-diphenyl-trichlorothane; PCB = polychlorinated biphenyl.

selected did not differ significantly at different cooling temperatures except for losses of highly volatile chemicals such as xylene (62%) which also had a high RSD (17%) at high cooling temperatures (20 °C). While, at lower cooling temperatures (−4 °C), the RSD of the all target chemicals ranged from 1.8 to 4.3% with over 93% extraction efficiency. The results indicate that cooling temperature is contributes greatly to the reproducibility and recovery of the analytes, and also, that low cooling temperatures are necessary for the high extraction efficiency of volatile chemicals. The simple semiconductor cooling system used here is adequate to reach a satisfactory low cooling temperature in the GP-MSE technique.

Dichloromethane, acetone and hexane are common extracting solvents in gas chromatographic sample treatment techniques. The trend in analytical chemistry is to minimize use of these toxic solvents in the sample analysis. The cooling system in the GP-MSE was therefore designed to minimize use of organic solvent. The amount of organic solvent consumed was measured at different cooling temperatures. Initial extracting solvent volume was fixed at 50 μL for each solvent. The vial heater temperatures were set at 150 and 300 °C; the cooler temperatures were set at 0 and −4 °C, and gas flow rates were set at 1, 3 and 5 mL min^{-1}. The data show that the amount of extracting solvent depends strongly on gas flow rate and solvent vapour pressure (Table 1). Dichloromethane, which is highly volatile, was most susceptible variation on the gas flow rate and cooling temperature. As shown in Table 1, of the three factors (heating temperature, cooler temperature and gas flow), gas flow rate is most important in affecting residual solvent volume. In the case of hexane, only 12.5 μL was lost under the conditions of heating temperature, cooling temperature, gas flow rate and experimental time at 300 °C, −4 °C, 5 mL min^{-1} and 10 min conditions, respectively. Overall, a low vapour pressure solvent, slow gas flow rate and low cooling temperature tend to minimize organic solvent.

3.5. Application

To examine the suitability of the automatic heating and cooling system for GC–MS quantitative determination of various kinds of analytes from real samples, some plant (such as poplar leaves) and biological (crucian carp) samples were sampled from the Changbai Mountain and Tumen River, and various target chemicals were spiked into the samples. The results of the GP-MSE approach were compared to these obtained from traditional Soxhlet extraction (SE). As shown in Table 2, the ratios of automatic GP-MSE and SE are close to 1 for most chemicals. Recoveries of "spiked" target analytes in the plant samples determined ranged from 90 to 97% for PAHs, from 89 to 95 for OCPs, from 87 to 112 for PCBs and from 88 to 96 for APs with the relative standard deviations (RSD) of <5%. For spiked biological samples, recoveries of the PAHs, OCPs, PCBs and APs ranged from 86 to 102% (RSD ≤ 6.2%), from 86 to 96% (RSD ≤ 5.8%), from 88 to 96 (RSD ≤ 6.9%), and from 83 to 94% (RSD ≤ 7.3%), respectively. In most case, the RSD% by GP-MSE was much lower than those by Soxhlet extraction. There is no doubt that the automatic electric heating device and semiconductor cooling device are very suitable for GP-MSE coupled with GC–MS analysis, for improved reliability and convenience.

4. Conclusion

The automatic heating and cooling system described here has improved the reliability and convenience of the GP-MSE technique. The system contributes to improved quantitative GC–MS determination of volatile and semivolatile chemicals from complex environmental matrices. A significant advantage of the new system is that the heater and condenser can be operated from a low-voltage power supply (e.g., a car battery) which allows the system to be used for on-site and on-line field sampling of volatile and semivolatile compounds from different sample matrices. Future studies on its applications to different volatile and semivolatile compounds (e.g., PAHs, APs, OCPs, essential oils) and from difference sample matrices (e.g., environment, drugs, foods, plants) are planned.

Acknowledgements

The authors acknowledge Dr. Richard Addison for his help in editing the manuscript. This work was supported by a grant from the National Natural Science Foundation of China (no. 21027009 and no. 21065014).

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