Journal of Hazardous Materials 159 (2008) 300–305

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Applicability of cloud point extraction coupled with microwave-assisted back-extraction to the determination of organophosphorous pesticides in human urine by gas chromatography with flame photometry detection

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ARTICLE INFO

Article history:
Received 24 August 2007
Received in revised form 12 February 2008
Accepted 12 February 2008
Available online 29 February 2008

Keywords:
Cloud point extraction
Organophosphorous pesticides (OPPs)
Microwave-assisted back-extraction
Gas chromatography with flame photometry detection (GC-FPD)
Human urine

ABSTRACT

A procedure for the determination of organophosphorous pesticides (OPPs) – phorate, diazinon, parathion-methyl, fenthion and quinalphos – in human urine was developed using the cloud point extraction of nonionic surfactant (Triton X-114) coupled with microwave-assisted back-extraction prior to gas chromatography with flame photometry detection (GC-FPD) analysis. The upper organic solution obtained from back-extraction was centrifugated simply for further cleanup for the sake of automatic injection. A preconcentration factor of 50 was obtained for these five OPPs extracted from only 10 mL of a sample. The limits of detection (LODs) were 0.07 ng mL^{-1} for phorate, fenthion and quinalphos, 0.04 ng mL^{-1} for diazinon and 0.08 ng mL^{-1} for parathion-methyl. The limits of quantification (LOQs) were 0.21, 0.12, 0.24, 0.21 and 0.21 ng mL^{-1}, respectively. Accuracy of the method was evaluated by bias, which ranged from +6.85 to −14.68%. Precision was also good; the relative standard deviations (R.S.D.s) were less than 9%. The method showed to be potential for biological monitoring.

1. Introduction

Pesticides have always been threats to human health due to their inherent toxicity. Organophosphorus pesticides (OPPs) are very toxic when absorbed by human organisms because of acetyl-cholinesterase deactivation. Studies showed that mixture of cholinesterase-inhibiting OPPs formulation adversely affected embryogenesis in mice which could have implications for susceptible population from occupational and environmental exposures [1]. In addition, OPPs were known to reduce the activity of neurotransmitters and hence to cause irreversible effects on the nervous system. Exposure to the hazardous pesticides is a concern of the general population, especially the agricultural population and residents living near industrial or contaminated areas. Pesticides have also been reported in patients with acute drug toxicity as a result of accidental or suicide-intended consumption [2,3]. The biological monitoring is to determine pesticides or their metabolites directly in biological fluids to measure exposure to these contaminants [4]. Hence, fast, accurate and sensitive analytical methods are necessary for the estimation of human exposure.

The determination of pesticides in human urine has been accomplished by means of solid-phase extraction (SPE) followed by high-performance liquid chromatography (HPLC) [5] and solid-phase microextraction (SPME) coupled with gas chromatography (GC) with nitrogen phosphorus detection (NPD) [6], flame photometry detection (FPD) [7] or mass spectrometer (MS) [8,9]. Though SPE and SPME avoid the use of large volumes of organic solvents, they have the disadvantages of relatively expensive special instruments and columns. Lately, a novel technique named cloud point extraction (CPE) has been used to extract pesticides from water samples prior to high-performance liquid chromatography (HPLC) [10,11], but extraction of pesticides from urine was scarce. Some researchers have applied CPE to extract ions from urine prior to atomic absorption spectrometry [12], capillary zone electrophoresis [13], and flow injection inductively coupled with plasma-optical emission spectrometry [14]. These imply that it is possible to apply CPE to extract pesticides from urine.

Cloud point extraction was introduced initially by Watanabe and co-workers in 1976 [15]. The cloud point refers to the phase separation of neutral surfactants including nonionic and zwitterionic surfactants induced by temperature. The result is the formation of two distinct phases: a surfactant-rich phase and an aqueous phase with concentration of surfactant close to the critical micellar concentration (cmc) [16]. This technique displays some advantages over liquid–liquid extraction (LLE), SPE and SPME, since it does

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doi:10.1016/j.jhazmat.2008.02.081

Table 1
Effect of incubation temperature

Pesticides	Recovery (%)			
	40 °C	50 °C	60 °C	70 °C
Phorate	76.9	101.8	98.3	64.7
Diazinon	88.2	105.2	101.6	89.3
Parathion-methyl	70.9	94.3	91.2	81.9
Fenthion	72.8	96.5	93.2	68.1
Quinalphos	73.4	92.1	91.0	69.9

not require the use of organic solvents; the volume of samples necessary for the analysis is lower; the surfactants used are less toxic and cheaper; and the extraction and preconcentration can be achieved in only one step. Because the surfactant-rich phase is compatible with hydroorganic mobile phases, CPE has been exploited to combine with HPLC, flow injection analysis (FIA) and capillary electrophoresis (CE). On the other hand, the application of CPE as a preconcentration step followed by gas chromatography is very rare. This is mainly because the viscous nature of the surfactant endangers the blocking of the capillary column. In 1997, Froschl et al. [17] have used two columns (silica and Florisil) to remove the surfactant prior to GC. Lately, Paleogos and co-worker [18] created a breakthrough on this problem. They applied microwaves or sonication to back-extract analytes from the surfactant-rich phase into a water-immiscible solvent prior to GC-flame ionization detection (FID) without any supplemental cleanup. Analogous back-extraction preceded GC analysis of UV-filters after CPE recently reported by Giokas et al. [19] and of diethylhexyladipate and acetyltributylcitrate by Kontominas and coworkers [20].

This paper described the development of CPE for the rapid and effective extraction and preconcentration of five OPPs from human urine coupled with GC-FPD by automated injection. The preconcentrated analytes were back-extracted from the obtained surfactant-rich phase into isooctane by short-term microwave application. The isooctane solution obtained from back-extraction was centrifuged for further cleanup and then directly injected into the GC.

The aim of this paper was to investigate the applicability of cloud point extraction coupled with microwave-assisted back-extraction to extract and preconcentrate organophosphorous pesticides from human urine prior to gas chromatography.

2. Experimental

2.1. Apparatus

The qualitative and quantitative analysis of the selected analytes were performed using an Agilent Technologies 6890N network GC System with a flame photometry detector (FPD) equipped with a 7683B series injector, 7683 series autosampler and a 30 m × 0.32 mm fused silica capillary column, coated with 0.25 μm film (HP-5, Agilent Technologies). The GC analysis of the selected analytes was carried out as follows: the column temperature was raised from 100 to 220 °C at 10 °C min^{-1}, then to 250 °C at 20 °C min^{-1}, and held for 3 min; the temperature of the injector was maintained at 230 °C while the detector was set at 250 °C. The determination was carried out in splitless mode.

Cloud point preconcentration was carried out in a HH.S11-2 water-bath (Beijing ChangAn Science Instruments, Beijing, China). A centrifuge (RJ-TDL-40B) from RuiJiang Instruments (Jiangsu, China) was used to separate the surfactant-rich phase from the aqueous phase. A LG microwave oven (700 W power, Tianjin, China) was used for back-extraction. A LX-100 palmate centrifuge for further cleanup and a vortex (QL-901) were purchased from Qilinbeier Apparatus Co. (Jiangsu, China).

2.2. Reagents

Phorate [CAS No. 298-02-2], diazinon [333-41-5], parathion-methyl [298-00-0], fenthion [55-38-9] and quinalphos [13593-03-8] (all with purity > 99.0%) were provided by the China Ministry of Agricultural Institute for Control of Agrichemicals. Stock standard solutions (~1000 mg L^{-1}) were made in ethyl acetate. Intermediary mixed standard solutions were prepared by diluting the stock standard solutions in ethyl acetate. Stock and intermediary mixed standard solutions were stored at 4 °C in the dark. Working mixed standard solutions (0.2 and 1.0 mg L^{-1}) were obtained everyday by diluting intermediary mixed standard solution (10 mg L^{-1}) with ethyl acetate. The nonionic surfactant Triton X-114 (TX-114) was purchased from Amresco (Ohio, USA) and used without further purification, to prepare a 100 g L^{-1} aqueous solution. All solvents used (isooctane, ethyl acetate) were of HPLC grade. HPLC purity water was obtained from milli-Q purification unit (Millipore, Bedford, MA, USA).

2.3. Urine storage

Urine specimen was collected from healthy, supposed non-exposed subjects. If used in 1 day, it would be stored in plastic containers at 4 °C without any preservatives. If not, it would be kept frozen at −20 °C until use. After the urine samples had been thawed, they were shaken for homogenization. The required volume was then sampled as quickly as possible to avoid sedimentation of any solids.

2.4. Extraction and preconcentration procedure

In a typical extraction experiment, 9 mL of urine was placed in a 15-mL screw-capped centrifuge tube. 50 μL of working mixed standard solution was added in it. And then, 140 μL of TX-114 stock solution (100 g L^{-1}), 1 mL of 1 M phosphate buffer pH 6.0, and 100 μL of saturated NaCl solution were subsequently added. The mixture was stirred for 2 min on the vortex and then left to stand for 15 min in the water bath at 50 °C.

The two phases were separated by centrifugation for 10 min at 4000 rpm. After cooled in ice bath, the surfactant-rich phase became viscous, and the upper aqueous phase was decanted by inverting the tube. 200 μL of isooctane was added and the preconcentrated analytes were extracted into the isooctane phase by applying microwaves (700 W) for 2 min. Two distinct layers were

Table 2
Analytical characteristics of the method

Pesticides	Retention time	Linear range (ng mL^{-1})	Calibration curve	Correlation coefficient	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	R.S.D. (%)
Phorate	9.072	0.10–20	A = 372.12C–27.243	0.9999	0.07	0.21	3.69
Diazinon	10.174	0.10–20	A = 148.57C–6.1381	0.9999	0.04	0.12	3.40
Parathion-methyl	11.093	0.10–20	A = 146.16C–27.891	0.9994	0.08	0.24	4.69
Fenthion	11.988	0.10–20	A = 165.22C–11.12	0.9999	0.07	0.21	3.92
Quinalphos	12.785	0.10–20	A = 231.59C–15.79	0.9999	0.07	0.21	3.72

Table 3
Precision and accuracy of this method for determining OPPs in human urine samples

Pesticides	Intra-day						Inter-day					
	1.00 ng mL^{-1}			5.00 ng mL^{-1}			1.00 ng mL^{-1}			5.00 ng mL^{-1}		
	Found (ng mL^{-1})[a]	R.S.D. (%)	Bias (%)	Found (ng mL^{-1})[a]	R.S.D. (%)	Bias (%)	Found (ng mL^{-1})[a]	R.S.D. (%)	Bias (%)	Found (ng mL^{-1})[a]	R.S.D. (%)	Bias (%)
Phorate	1.02 ± 0.08	7.46	+2.17	4.77 ± 0.11	2.33	−4.67	1.07 ± 0.06	5.51	+6.85	4.60 ± 0.21	4.48	−8.01
Diazinon	0.95 ± 0.05	5.66	−4.67	4.64 ± 0.19	4.05	−7.27	0.98 ± 0.06	5.89	−2.24	4.55 ± 0.13	2.76	−8.97
Parathion-methyl	0.94 ± 0.08	8.94	−5.67	4.53 ± 0.13	2.82	−9.43	0.93 ± 0.08	9.02	−7.19	4.35 ± 0.32	7.33	−12.99
Fenthion	1.00 ± 0.05	5.33	−0.33	4.51 ± 0.15	3.27	−9.83	1.01 ± 0.08	7.72	+1.27	4.40 ± 0.13	3.01	−12.03
Quinalphos	0.89 ± 0.05	5.93	−11.50	4.34 ± 0.08	1.81	−13.30	0.85 ± 0.06	6.74	−14.68	4.30 ± 0.21	4.99	−13.93

[a] Average value ± S.D.

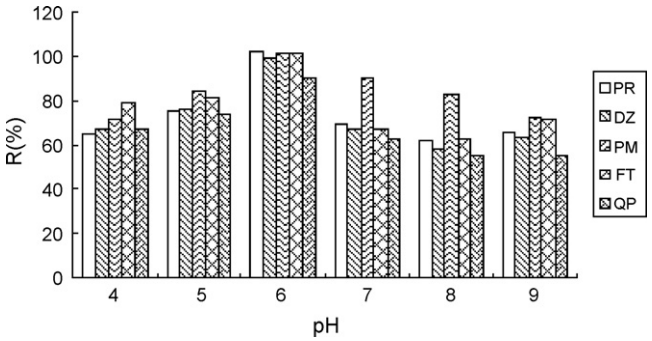

Fig. 1. The effect of pH on extraction recovery conditions: 1.4 g L^{-1} TX-114, 100 μL saturated NaCl solution, 1.00 ng mL^{-1} of each OPPs, 200 μL isooctane, microwave at 700 W for 2 min.

formed: the surfactant-rich phase (less than 200 μL, lower) and the isooctane phase (200 μL, upper). The upper phase was carefully transferred to a 1.5-mL EP vial with a 200-μL DRAGON MED pipettor, and then centrifugated for 1 min at 3000 rpm for further cleanup. The clear isooctane solution was then transferred to autosampler vial and analyzed by GC-FPD through automatic injection. Because of automatic injection, the study adopted the external standard method to avoid the internal standard.

3. Results and discussion

3.1. Effect of pH

Cloud point extraction of these five OPPs was performed in different pH buffer solutions. 1 mL of 1 M phosphate buffer solution (pH range from 3 to 9) was added to study the influence of pH on their extraction recoveries. From Fig. 1, it can be seen that the extraction efficiency was almost quantitative (approximately 100%) at pH 6, while at other pH values the recoveries were almost constant. Since, in CPE procedures the extraction efficiency is almost independent on the pH for the hydrophobic, non-ionizable nature of the analytes, but this fact could not explain the observed results. These five OPPs can hydrolyze in acid and alkaline media and they are stable at pH 6–7. In addition, from a medical point of view, for a healthy person, the normal pH values of urine range from 4.6 to 8.0 under common diet condition and the mean value is 6.0. So it is inferred that the pH selected was related to the stability of OPPs and the inherent pH of urine. Due to the numerous influential factors for the inherent pH of urine, it is essential to adjust the pH of the solution. In addition, the influence of the buffer amount was assayed. The results showed that no obvious variation took place in the extraction yield. Therefore, 1 mL of 1 M phosphate buffer solution (pH 6) was chosen for further study.

3.2. Effect of surfactant concentration

The amount of surfactant should be sufficient for the quantitative extraction of the target analytes, but not excessive to interfere with the back-extraction process, so it is important to optimize its amount. It can be seen from Fig. 2 that a volume of 120–150 μL of the 100 g L^{-1} stock TX-114 solution (corresponding to 1.2–1.5 g L^{-1} final surfactant concentration) produced optimal recoveries. Outside this optimal range, smaller amounts led to low recoveries because the surfactant was not sufficient to make reproducible measurements of extraction and separation. However, if surfactant concentration was increased from the recommended, the extraction recoveries were much higher than 100%. The reason may be that the volume of the surfactant-rich phase was much

Table 4
Comparison the performance of this method with that of other reported methods for extracting the studied OPPs from urine

Extractions	Analysis	OPPs	Linear range (ng mL^{-1})	Recovery (%)	LODs (ng mL^{-1})	References
SPME	GC-FPD	Diazinon/parathion-methyl/fenthion	1–100	82–140	0.1–0.4	[7]
SPME	GC–MS	Quinalphos	10–500, 500–50,000	24.5–27.7	2	[9]
CPE–back extraction	GC-FPD	Diazinon/parathion-methyl/fenthion/quinalphos	0.10–20	85–107	0.04/0.08/0.07/0.07	This method

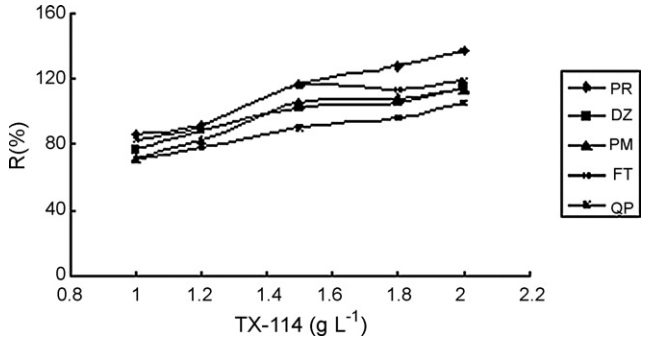

Fig. 2. The effect of surfactant concentration on recovery conditions: 1 mL of 1 M phosphate buffer pH 6.0, 100 μL saturated NaCl solution, 1.00 ng mL^{-1} of eachOPPs, 200 μL isooctane, microwave at 700 W for 2 min.

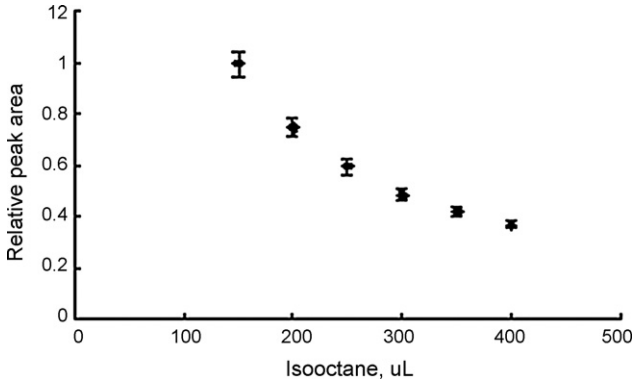

Fig. 3. The effect of isooclane volume on the analytical response. Conditions: 1 ml of 1 M phosphate buffer pH 6.0, 100 μL saturated NaCl solution, 1.00 ng mL^{-1} of each OPPs, microwave at 700 W for 2 min. The relative peak area is calculated by dividing the largest value of 150 μL of isoociane into the other individual values.

larger than 200 μL, and consequently turbid substance appeared at the interface of isooctane and surfactant after microwave-assisted back-extraction. The optimal surfactant concentration was chosen as 1.4 g L^{-1} in this paper, which can ensure to achieve optimal recoveries of the target analytes and avoid disturbing the back-extraction process.

3.3. Effect of Incubation temperature and duration

Incubation time and temperature also play roles in the CPE process as other parameters. It is reported that increasing the temperature or the equilibration time can decrease the amount of water in a surfactant-rich phase and hence the volume of that phase decreases. This is because dehydration between the hydrogen bonds occurs when increasing temperature [21]. This phenomenon that the volume of the surfactant-rich phase decreases has advantage to the back-extraction process in our study. Here, keeping incubation time 15 min constant, incubation temperature varied between 40 and 70 °C was examined. The results, listed in Table 1, showed that the optimum recoveries were obtained at 50 and 60 °C. During the experiment, it was found that the volume of the surfactant-rich phase decreased with increasing incubation temperature, which was the same as described in Ref. [21]. At the same time, flaxen powder which is the precipitate of urine proteins appeared in urine and the amount was related to temperature. After centrifugation, the precipitate was at the bottom of the tube and the amount was not enough to disturb the back-extraction. It is desired that the amount of the precipitate could be as little as possible. Therefore, a compromise should be reached. Furthermore, some of these OPPs are unstable at high temperature. For example, diazinon can decompose when the temperature is higher than 50 °C. So incubation temperature at 50 °C was selected for further study. Secondly, different equilibration time ranging from 5 to 25 min at 50 °C was tested. The results showed that this variety practically did not affect the recoveries of the analytes and the data were not listed here. Hence, duration was chosen as 15 min in this work.

3.4. Microwave-assisted back-extraction

Among the common water immiscible solvents, the volatility of isooctane is the weakest, and Refs. [18,20] reported isooctane can provide good reproducibility when the microwave approach was applied. So isooctane was selected as back-extraction reagent. In this paper, microwaves were applied to back-extract the analytes from the surfactant-rich phase into isooctane. It is found that when the volume of isooctane was 200 μL and the power of microwaves was 700 W, the five OPPs were quantitatively extracted into isooctane within 2 min in a domestic microwave oven. Further application of microwaves up to 4 min gave no significant enhancement in the analytical response. And the two phases (isooctane and surfactant-rich phase) were separated completely. Therefore, a 2-min application was selected for further study.

There are two important factors to be taken into account in optimizing the volume of isooctane: the preconcentration factor and the required volume of the automated injection. As can be seen from Fig. 3, the analytical response was relatively high and the volume also satisfied the automated injection when 200 μL of isooctane was used. Outside this optimal point, larger volumes resulted in a gradual decrease of the peak area (i.e. reduction of the preconcentration factor) due to subsequent dilution, while smaller amounts which seemed to yield higher preconcentration factor often produced emulsion or slurries, thus leading to poor reproducibility. So a volume of 200 μL was finally selected as the optimum and the preconcentration factor calculated theoretically was 50 for these five OPPs extracted from only 10 mL of a sample.

3.5. Centrifugation cleanup

During the experiment, it is found that the upper isooctane phase was transparent after microwave-assisted back-extraction but a few minutes later the phase was usually (but not always) no longer clear. In order to avoid blocking of the capillary column and achieving automated injection successfully, centrifugation was adopted for further cleanup. After microwave back-extraction, the upper isooctane phase was immediately transferred to a 1.5-mL EP vial with a pipettor, and then centrifugated for 1 min at 3000 rpm to obtain the clear isooctane solution.

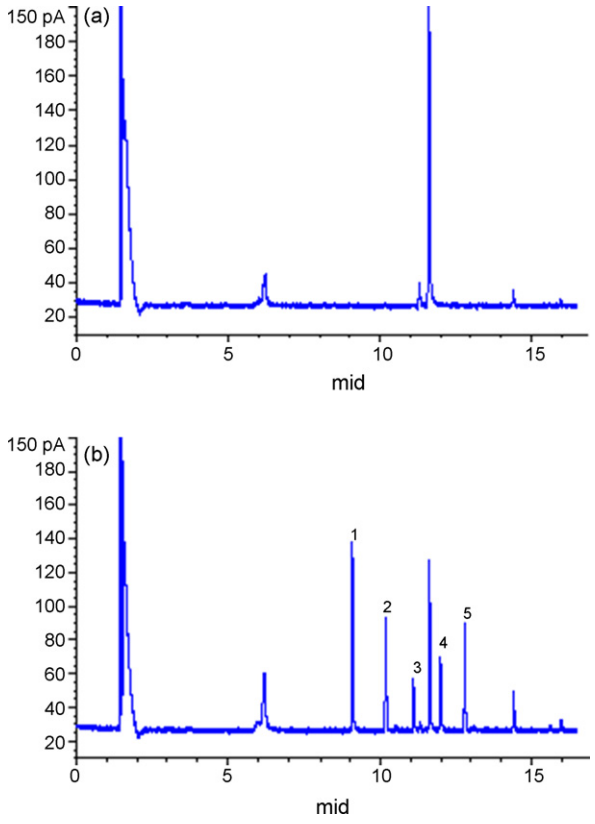

Fig. 4. Chromatogram of a (a) blank urine sample and (b) urine sample spiked with 1.00 ng mL^{-1} of each OPPs (l: phorate; 2: diazinon; 3: parathion-methyl; 4: fentluon; 5: quinarphos) after the extraction procedure. Conditions as mentioned in the text.

3.6. GC-FPD analysis

Cloud point extraction coupled with microwave-assisted back-extraction has been combined with GC-FID successfully [18–20]. In this paper, we want to apply this technique to GC-FPD with automatic injection. According to the optimal extraction procedure, a blank urine sample and a urine sample spiked with 1.00 $\mu g\,L^{-1}$ of each OPPs were subjected to the CPE procedure. The chromatograms were shown in Fig. 4. Due to high selectivity of FPD only to the compounds containing phosphorus and sulfur element, the blank chromatogram (Fig. 4a) presented a smooth baseline, which was much better than that of FID. Some peaks in it may result from the phosphate buffer. As can be seen from Fig. 4b, the peaks of these five OPPs appeared free from any interference and their shapes were acceptable and allowed precise quantification. In addition, repeated injections of the blank and spiked extract also gave no significant fluctuations of the retention times.

3.7. Analytical characteristics of the method

3.7.1. Calibration curves and limits

The calibration curves were prepared by mixed standard solutions. The peak areas for the five OPPs were proportional to their concentrations (ng mL^{-1}) in good linear relationships. The relative standard deviations ranged between 3.40 and 4.69%. The limit of detection (LOD) of each OPP was calculated based on a signal-to-noise ratio 3:1. The limit of quantification (LOQ) was defined as three times the limit of detection. The results were listed in Table 2.

3.7.2. Precision and accuracy

In order to support the validation of a method, International Union of Pure and Applied Chemistry (IUPAC) proposed four approaches to obtain reference values for trueness experiments. They are certified reference materials (CRMs), reference materials, use of a reference method and use of spiking/recovery [22]. Because (certified) reference materials were unavailable, we selected the use of spiking/recovery to evaluate the accuracy of the proposed method.

A less costly and very commonly applied expedient, is to estimate the recovery of the analyte added as a spike in a separate experiment. The added analyte acts as a surrogate for the native analyte. However, if the added analyte is not so firmly bound to the matrix as the native analyte, the surrogate recovery will tend to be high in relation to that of the native analyte. The phenomenon may appear in the solid matrix. Because the matrix used in this paper is liquid, the disadvantages can be neglected. In common, the precision of a method is evaluated by the R.S.D. of replicate measurements. The values should be within ±15% at all concentrations. In this paper, precision was evaluated at 1.00 and 5.00 ng mL^{-1} for fortified human urine analyzing six replicates for each concentration in the same day (intra-day precision) and on three different days (inter-day precision). These data were less than 10%, which indicated that the method was reproducible. The accuracy of the method was expressed as bias. The values were listed in Table 3. It can be seen that the accuracy of this method was acceptable.

3.8. Comparison of this method with others

Till now, the methodologies which have been reported for extracting the studied five OPPs from urine are mostly SPME. Table 4 summarized the comparison between the performance of the proposed method and that of other reported methods for extracting the studied OPPs from urine. Compared with SPME, the proposed method only need some cheap surfactant and does not require special instrument. The LODs of this method are lower than the others. The linear range is narrower because 20 ng mL^{-1} is not the largest concentration of the linear range. If wide linear range is required in practice we can make it much wider. The instruments for SPME are not furnished in our laboratory so it is a pity that the reported method [7,9] cannot be regarded as a reference method to further validate the proposed method. Due to not obtaining the real urine samples from the person who has been exposed to the contaminated environment, therefore, we only compared the performance of the proposed method with others reported simply to indicate its simpleness, cheapness and convenience.

4. Conclusion

In this study, cloud point extraction coupled with microwave-assisted back-extraction to the determination of OPPs in human urine by GC-FPD with automated injection was successfully developed at low nanogram per milliliter level. The upper isooctane phase obtained from back-extraction was centrifugated for further cleanup to achieve automatic injection. It demonstrates that this method is cheaper and easier than SPE and SPME. The performance of the experiment indicates that it can be used as a rapid, easy, sensitive, convenient and practical method for biological monitoring and the estimation of human exposure.

References

[1] J. Gomes, S.V. Anilal, O. Lloyd, Reproductive and developmental toxicity from organophosphorus pesticide formulations: litter size and low fetal weight, J. Agric. Safety Health 5 (1999) 239.

[2] M. Maroni, G. Catenacci, D. Galli, D. Cavallo, G. Ravazzani, Biological monitoring of human exposure to acephate, Arch. Environ. Contam. Toxicol. 19 (1990) 782.
[3] K.T. Maddy, J.B. Knaak, D.B. Gibbons, Monitoring the urine of pesticide applicators in California for residues of chlordimeform and its metabolites 1982–1985, Toxicol. Lett. 33 (1986) 37.
[4] C. Aprea, G. Sciarra, P. Sartorelli, E. Desideri, R. Amati, E. Sartorelli, Biological monitoring of exposure to organophosphorus insecticides by assay of urinary alkylphosphates: influence of protective measures during manual operations with treated plants, Int. Arch. Occup. Environ. Health 66 (1994) 333.
[5] G. Carlucci, D.D. Pasquale, F. Ruggieri, P. Mazzeo, Determination and validation of a simple high-performance liquid chromatographic methods for simultaneous assays of iprodione and vinclozolin in human urine, J. Chromatogr. B 828 (2005) 108.
[6] H. Tsoukali, G. Theodoridis, N. Raikos, I. Grigoratou, Solid phase microextraction gas chromatographic analysis of organophosphorus pesticides in biological samples, J. Chromatogr. B 822 (2005) 194.
[7] F.J. López, E. Pitarch, S. Egea, J. Beltran, F. Hernández, Gas chromatographic determination of organochlorine and organophosphorous pesticides in human fluids using solid phase microextraction, Anal. Chim. Acta 433 (2001) 217.
[8] E. Gallardo, M. Barroso, C. Margalho, A. Cruz, D.N. Vieira, M. LÓpez-Rivadulla, Solid-phase microextraction for gas chromatographic/mass spectrometric analysis of dimethoate in human biological samples, Rapid Commun. Mass Spectrom. 20 (2006) 865.
[9] E. Gallardo, M. Barroso, C. Margalho, A. Cruz, D.N. Vieira, M. LÓpez-Rivadulla, Determination of quinalphos in blood and urine by direct solid-phase microextraction combined with gas chromatography–mass spectrometry, J. Chromatogr. B 832 (2006) 162.
[10] R. Carabias Martínez, E. Rodríguez Gonzalo, M.G. García Jiménez, C. García Pinto, J.L. Pérez Pavón, J. Hernández Méndez, Determination of the fungicides folpet, captan and captafol by cloud-point preconcentration and high-performance liquid chromatography with electrochemical detection, J. Chromatogr. A 754 (1996) 85.
[11] R. Halko, C. Padron Sanz, Z. Sosa Ferrera, J.J. Santana Rodríguez, Determination of benzimidazole fungicides by HPLC with fluorescence detection after micellar extraction, Chromatographia 66 (2004) 151.
[12] J.L. Manzoori, G. Karim-Nezhad, Sensitive and simple cloud-point preconcentration atomic absorptionspectrometry: application to the determination of cobalt in urinesamples, Anal. Sci. 19 (2003) 579.
[13] C. Ortega, S. Cerutti, R.A. Olsina, L.D. Martínez, M.F. Silva, Simultaneous determination of dysprosium and iron in urine by capillary zone electrophoresis coupled to cloud point extraction, J. Pharmaceut. Biomed. Anal. 36 (2004) 721.
[14] C. Ortega, M.R. Gomez, R.A. Olsina, M.F. Silva, L.D. Martinez, On-line cloud point preconcentration and determination of gadolinium in urine using flow injection inductively coupled plasma optical emission spectrometry, J. Anal. Atom. Spectrom. 17 (2002) 530.
[15] J. Miura, H. Ishii, H. Watanabe, Bunseki Kagaku 25 (1976) 808.
[16] N.D. Gullickson, J.F. Scamehom, J.H. Harwell, in: J.F. Schamehom, J.H. Harwell (Eds.), Surfactant-based Separation Processes, Marcel Dekker, New York, 1989, pp. 139–151.
[17] B. Froschl, G. Stangl, R. Niessner, Combination of micellar extraction and GC-ECD for the determination of polychlorinated biphenyls (PCBs) in water, Fresen. J. Anal. Chem. 357 (1997) 743.
[18] T.I. Sikalos, E.K. Paleologos, Cloud point extraction coupled with microwave or ultrasonic assisted back extraction as a preconcentration step prior to gas chromatography, Anal. Chem. 77 (2005) 2544.
[19] D.L. Giokas, V.A. Sakkas, T.A. Albanis, D.A. Lampropoulou, Determination of UV-filter residues in bathing waters by liquid chromatography UV-diode array and gas chromatography-mass spectrometry after micelle mediated extraction-solvent back extraction, J. Chromatogr. A 1077 (2005) 19.
[20] P.D. Zygoura, E.K. Paleologos, K.A. Riganakos, M.G. Kontominas, Determination of diethylhexyladipate and acetyltributylcitrate in aqueous extracts after cloud point extraction coupled with microwave assisted back extraction and gas chromatographic separation, J. Chromatogr. A 1093 (2005) 29.
[21] Z. Sosa Ferrera, C. Padrón Sanz, C. Mahugo Santana, J.J. Santana Rodríguez, The use of micellar systems in the extraction and pre-concentration of organic pollutants in environmental samples, Trends Anal. Chem. 23 (2004) 469.
[22] M. Thompson, S.L.R. Ellison, R. Wood, Harmonized guidelines for single-laboratory validation of methods of analysis, Pure Appl. Chem. 74 (2002) 835.