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Application of liquid-phase microextraction and gas chromatography–mass spectrometry for the determination of polychlorinated biphenyls in blood plasma

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Abstract

This study investigated the feasibility of applying liquid-phase microextraction combined with gas chromatography–mass spectrometry (GC–MS) to determine polychlorinated biphenyls (PCBs) in blood plasma. An efficient and simple extraction technique has been developed for the enrichment of PCBs from human blood plasma samples using single-step liquid-phase microextraction (LPME) in conjunction with a hollow fibre membrane (HFM). An eight PCB congener mixture was spiked into 2.5 ml of blood plasma, and the solution was then adjusted to pH 10.5 with a salinity of 20% (w/v) prior to making the total volume to 5 ml with ultrapure water. The porous HFM, filled with 3 μl of organic solvent, was then immersed into the solution, which was continuously agitated at 700 rpm for 30 min. Extract (1 μl) containing the pre-concentrated analytes was then injected into a GC–MS without further pre-treatment. Using an optimised extraction procedure, a large enrichment factor of the analytes, i.e. up to 241-fold was achieved in 30 min. The procedure resulted in a relative standard deviation of <11% ($n = 6$), and a linear calibration range from 2.5 to 150 μg/l ($r > 0.999$), and detection limits between 0.07 and 0.94 μg/l, respectively. To demonstrate the feasibility of the procedure, PCB concentrations were determined in actual blood samples collected from the local population in Singapore using the optimised LPME technique.
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Keywords: Liquid-phase microextraction; Polychlorinated biphenyls

1. Introduction

Polychlorinated biphenyls (PCBs) are potent environmental contaminants due to their propensity to accumulate in biological tissues, and their persistence and ubiquity in the global environment [1–3]. Many publications have specifically identified and quantified PCB congeners in human milk, adipose tissue and blood samples [4–7]. PCBs readily accumulate in the food chain, especially in meat, fish, and dairy products due their lipophilic nature and low water solubility. It is now evident that PCBs are transferred from mother to foetus and new-born babies via blood exchange placenta and to new-born infants via breast milk [8–11]. Trace amounts of PCB congeners have also been associated

with endocrine disruption and a higher incidence of foetal miscarriage [12].

Quantitative analysis of trace level concentrations of PCBs in biological matrices including blood, is a significant challenge demanding an effective sample preparation procedure prior to analysis. Currently, there are three principal methods of extraction of PCBs from blood samples: liquid–liquid extraction (LLE) [13], solid-phase extraction (SPE) [14] and solid-phase microextraction (SPME) [15]. The first two methods suffer from several problems including the relatively large volume of sample and extraction solvent required and the need for multi-step sample extraction and clean-up procedures [16]. PCBs extracted by SPME suffer extensively from analyte carry-over and incomplete analyte desorption, resulting in unacceptable quantification errors of up to 20% [17]. New solvent-minimised techniques based upon liquid-phase microextraction (LPME), as an alternative to liquid–liquid extraction, have been developed. Compared to LLE, LPME is faster and less expensive as

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only a few microliters of sample and solvent are required [18–20]. For single drop LPME techniques, maintenance of the solvent drop prior to analysis is a particularly challenging task in complex matrices [21]. A more advanced LPME technique supported by a disposable hollow fibre membrane (HFM) has proved to be effective for the clean-up and enrichment of analytes in complex matrices including soil slurry [22], urine and plasma [23,24]. LPME supported by an HFM two-phase system is compatible with GC analysis. Three-phase systems are suited to capillary liquid chromatography [25], flow injection analysis tandem mass spectrometry [26] and capillary electrophoresis [27] due to the aqueous acceptor phase. LPME using HFM eliminates the analyte carry-over effect since the HFM is used only once. Due to its simplicity, rapid, reproducibility and reliability LPME can be used for routine analysis of analytes extracted from biological matrices.

The purpose of this work was to develop a simple, efficient single-step extraction method for the screening and quantitative determination of PCBs in human blood plasma. Analytical parameters investigated included: the type of solvent used; sample extraction; time; pH and stir speed. The optimised method is proposed as an efficient alternative to more expensive, time consuming conventional methods.

2. Experimental section

2.1. Standard and reagents

A US Environmental Protection Agency (EPA) standard 521, 521.5 PCB mixture of eight congeners (500 μg per congener ml^{-1}) was used as a model standard for technique optimisation (Aldrich, Milwaukee, WI, USA). HPLC grade solvents were purchased from Merck (Darmstadt, Germany), and ultrapure water was obtained from a Milli-Q system (Millipore, Milford, MA, USA). Sodium phosphate tribasic (Na$_3$PO$_4$·12H$_2$O) crystals (93% pure, Mallinckrodt, Paris, KY, USA) were used to prepare the buffer by dissolving 23.8 g of Na$_3$PO$_4$·12 H$_2$O into 250 ml of water; giving a final pH of 13 [28]. The PCB standard was diluted in acetone at a concentration of 10 μg ml^{-1}. Accurel Q3/2 polypropylene hollow fibre (Membrana, Wuppertal, Germany) with an inner diameter of 600 μm, a wall thickness of 200 μm and a pore size of 0.2 μm was used for PCB extraction.

2.2. Sample collection and preparation

Blood samples were randomly obtained from the National University Hospital, Singapore. Samples comprised blood from Chinese, Malay and Indian adult individuals (six samples each group). Blood plasma was prepared by centrifugation of whole blood samples and then stored at −20 °C prior to analysis. Sample pH was adjusted to 10.5 by adding phosphate buffer and the ionic strength of the sample solution was increased by adding 15% of NaCl (w/v). All glass-
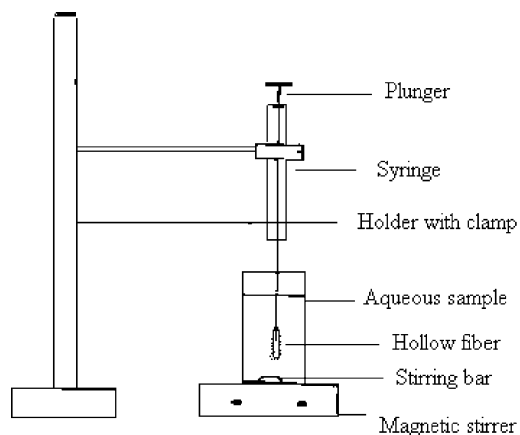

Fig. 1. Schematic set-up of liquid-phase microextraction supported by hollow fibre membrane.

ware and magnetic stirring bars were washed with detergent, soaked overnight in dilute nitric acid, rinsed with deionized water and acetone before drying at 105 °C.

2.3. Liquid-phase microextraction

A 10 μl microsyringe, with a cone tip (0.47 mm o.d.) (Hamilton, Reno, NV, USA) was used for PCB extraction from blood samples. Before each extraction, the syringe was rinsed with acetone and then toluene a total of 10 times to avoid analyte carry-over and air bubble formation. Finally, 3 μl of toluene was drawn into the syringe. The syringe needle was then tightly fitted with a 1.2 cm length of HFM which was then impregnated with toluene for 10 s to open membrane pores prior to immersion 5 mm below the surface of a sample solution in a 5 ml volumetric flask. The syringe plunger was depressed completely so that the HFM was completely filled with toluene. Fig. 1 shows a schematic diagram of the LPME set-up. PCB extraction from the sample solution to the solvent within the HFM was undertaken over a period of 30 min under a magnetic rotation speed of 700 rpm. Following sample extraction, the stirrer was switched off and the extract in the hollow fibre was retracted into the syringe. The hollow fibre was detached and discarded. The volume of the extract was adjusted to 1 μl to remove the residual sample solution; this was injected into the GC–MS for analysis.

2.4. GC–MS analysis

Sample analysis was conducted on a Shimadzu (Tokyo, Japan) QP5050 GC–MS system equipped with a Shimadzu AOC-20i auto sampler and DB-5 fused silica capillary column (30 m × 0.32 mm internal diameter and 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas with a flow rate of 1.5 ml min^{-1} and a split ratio of 20. The injection and interface temperature was 250 and 280 °C, respectively. The GC temperature

Table 1
Analytical GC–MS-selective ion monitoring (SIM) conditions of PCB congeners with octanol–water partition coefficient (log K_{ow}) values

Congeners	Retention time (min)	Primary ion (m/z)	Confirmation ion (m/z)	log K_{ow}[a]
2-Chlorobiphenyl (CB-1)	14.5	188	152, 154	4.3
2,3-Dichlorobiphenyl (CB-5)	17.1	222	224, 152	4.8
2,4,5-Trichlorobiphenyl (CB-29)	18.7	258	186, 256	5.4
2,2′,4,4′-Tetrachlorobiphenyl (CB-47)	19.9	290	292, 220	5.9
2,2′,3′,4,6-Pentachlorbiphenyl (CB-98)	21.1	326	254, 184	6.5
2,2′,4,4′,5,6′-Hexachlorobiphenyl (CB-154)	22.3	360	290, 362	7.1
2,2′,3,3′,4,4′,6-Heptachlorobiphenyl (CB-171)	24.8	394	396, 252	7.6
2,2′,3,3′,4,5′,6,6′-Octachlorobiphenyl (CB-200)	24.9	358	430, 360	8.2

[a] [33].

programme was as follows: initial temperature 50 °C held for 2 min, then an increase at 10 °C min^{-1} to 300 °C, held for 3 min. One microliter of sample was injected in splitless mode with an injection time of 2 min. Samples were analysed in selective ion monitoring mode (SIM) mode with a detector voltage of 1.5 kV and a scan range from m/z 50 to 500. A specific ion was selected for each PCB congener and the most abundant ion was selected as the quantitative ion, while two other ions were used for confirmation of individual PCB congeners (Table 1).

2.5. Enrichment factor and recoveries

In two-phase LPME, the analytes were extracted from the aqueous sample and organic solvent present in the pores and inside the lumen of the HFM. This process can be illustrated as follows [22]:

$$A_{\text{(aqueous sample)}} \leftrightarrow A_{\text{(organic solvent)}}$$

The enrichment factor E_f for a diluted, i.e. 5 ml sample and 3 μl of extraction solvent was calculated based on the equation

$$E_f = \frac{1}{(V_o/V_a + 1/K)}$$

In this equation K is the distribution co-efficient, V_o the volume of organic solvent and V_a is the volume of aqueous sample. K is calculated as follows:

$$K = \frac{C_{o\,eq}}{C_{a\,eq}}$$

where $C_{o\,eq}$ is the concentration of analyte in the organic phase and the $C_{a\,eq}$ is the concentration of analyte in the aqueous phase. The maximum enrichment factor value is equal to the analyte distribution coefficient between the extraction solvent and the sample, where a large distribution coefficient represents a higher extraction recovery. As for SPME, LPME is also an equilibrium extraction procedure. Relative, rather than absolute, recoveries of the analytes were thus calculated. The relative recovery is defined as the ratio of the peak areas of the analytes in plasma and ultrapure water sample extracts, with both samples spiked at the same concentration levels of PCBs.

3. Results and discussion

3.1. Method evaluation

Blank blood plasma extraction was carried out prior to spiking with the mixture of PCB congeners. Fig. 2a shows an extraction chromatogram from a blank sample of human blood plasma. PCBs were not detected in this sample and it was therefore used for subsequent method evaluation. Fig. 2b shows an extraction chromatogram of the same blood plasma sample spiked with 40 μg/l of the PCB mixture. It can be noted that matrix peaks present in the blank blood plasma extraction do not interfere with the studied PCB compounds. Factors affecting the LPME procedure including pH, salt content, stirring rate, extraction solvent, volume of solvent used and extraction time were optimised in order to minimise analysis time and maximise PCB congener extraction recovery. Initially, a raw blood plasma sample with no dilution was used, but this resulted in a poor enrichment factor being achieved for all PCB congeners analysed. However, sample dilution improved the extraction precision, efficiency, detection limit and extraction yields. It is likely that sample dilution reduces the extent of interfering substances blocking the pores of the HFM, with the result that the extraction is more efficient and consistent. The enrichment factor was determined by measuring GC peak area before and after LPME. Optimisation of analytical parameters and enrichment factors was obtained by undertaking sample analysis in triplicate. The LPME technique resulted in an enrichment factor of between 67 and 241. This is higher than that achieved in previous studies using liquid-membrane extraction and microporous membrane liquid–liquid extraction which typically gives an enrichment factor of between 30 and 70 for biological samples [29–31]. LPME also has the advantage of requiring a relatively simple extraction set-up with only a few microliters of solvent and sample needed, with no further pre-treatment required prior to analysis.

3.2. Effect of extraction time

The effect of extraction time on PCB congener extraction efficiency was evaluated over 5, 10, 20, 30, and 60 min. A typical mass diffusion (extraction) profile of the analyte
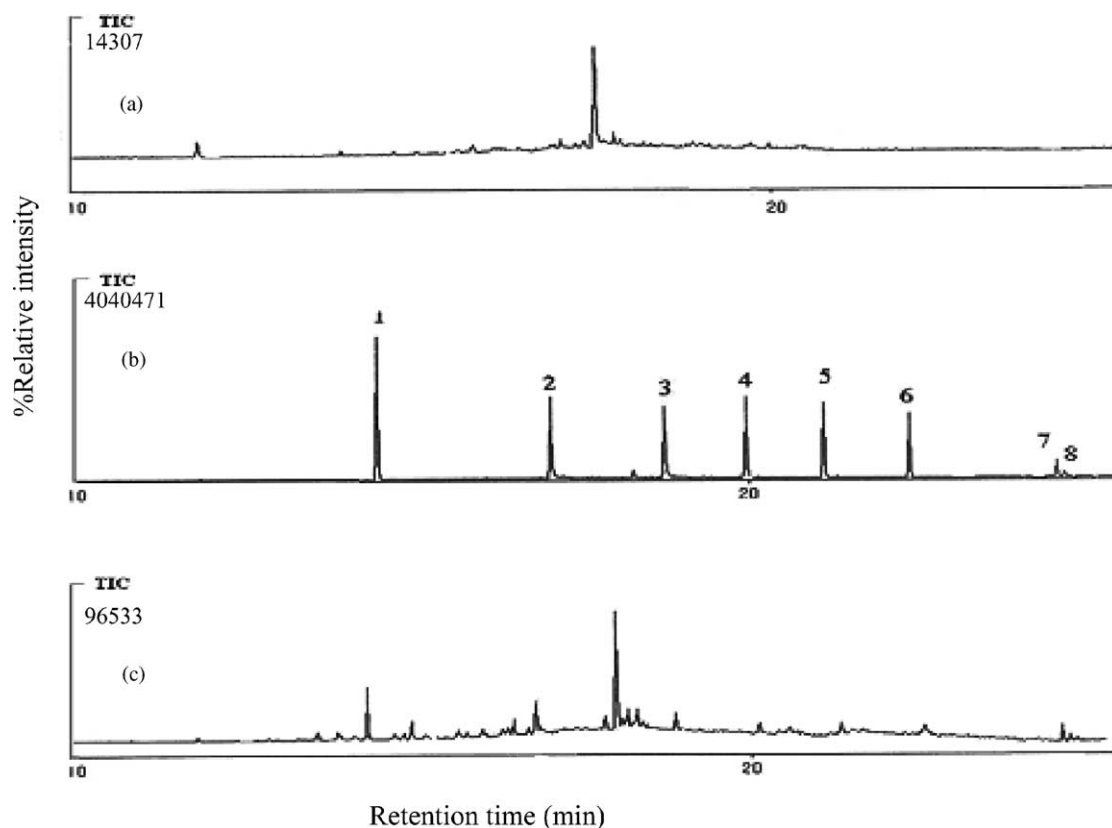
Fig. 2. GC–MS of: (a) blank plasma extract; (b) extract of plasma spiked with 40 μg/l of each PCB congener and (c) real sample extract [1] CB-1 [2] CB-5 [3] CB-29 [4] CB-47 [5] CB-98 [6] CB-154 [7] CB-171 [8] CB-200. GC–MS conditions as in the text.

between the sample and extraction solvent consisted of an initial rapid partitioning phase followed by a slower prolonged uptake, and then a steady-state equilibrium. After reaching equilibrium, the amount of analyte extracted becomes a theoretical constant when plotted against extraction time [32]. Fig. 3 shows that greater peak areas were obtained for PCB congeners with an increasing extraction time up to 30 min after which equilibrium was achieved. As such, 30 min was selected as the optimum extraction time for sam-

ple analysis as all congeners reached equilibrium within this period.

3.3. Selection of solvent

The selection of the best extraction solvent is a prerequisite in order to achieve the highest analyte enrichment factor as properties including analyte solubility and solvent volatility affect extraction efficiency. The HFM used for
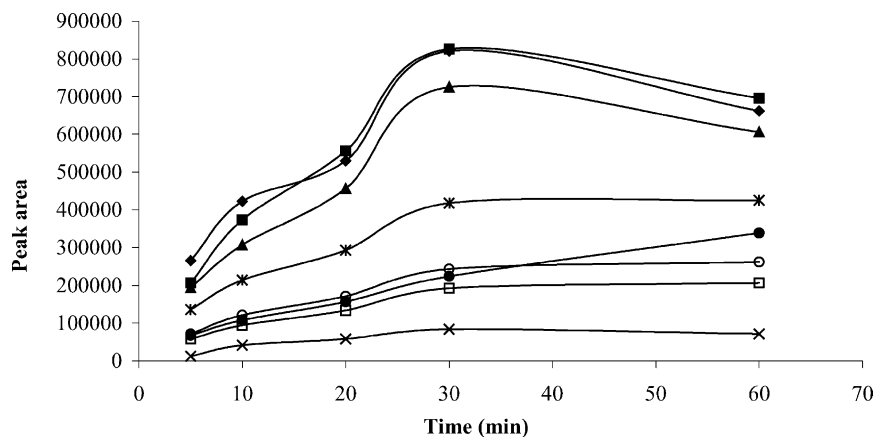

Fig. 3. Equilibration profile of PCB congeners during liquid-phase microextraction of blood plasma spiked with 20 μg/l of each PCB congener: (◆) CB-1; (■) CB-5; (▲) CB-29; (×) CB-47; (✶) CB-98; (●) CB-154; (○) CB-171; (□) CB-200.
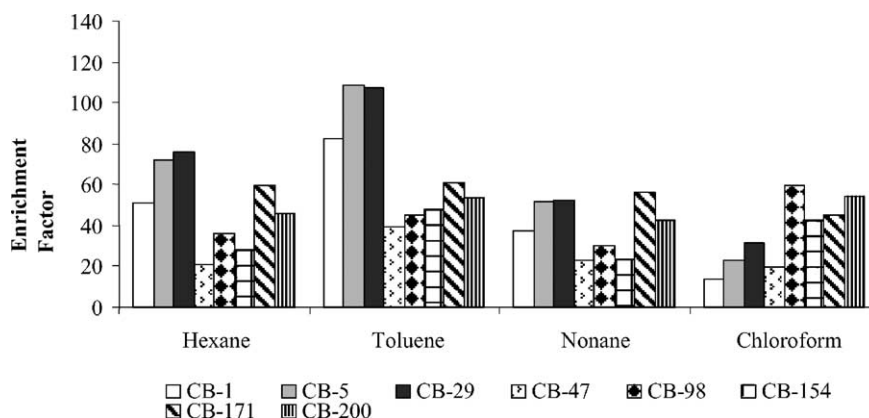
Fig. 4. Extraction efficiency using different organic solvents ($n = 3$). Blood plasma samples were spiked at 40 μg/l of individual PCB congeners. Extraction solvent volume: 3 μl.

experimentation is made of polypropylene and the solvent should, therefore, have a polarity that effectively matches that of the HFM. High polarity solvents are not suitable for LPME due to solvent leakage via the HFM pores [24]. Four solvents were selected for study, according to decreasing polarity, i.e. chloroform, toluene, *n*-hexane and *n*-nonane. The PCB congeners analysed are hydrophobic compounds with log values of octanol–water partition coefficients (K_{ow}) ranging from 4.3 to 8.2 [33] (see Table 1). As such, congeners were expected to readily partition into the solvent. Toluene gave higher enrichment factors (see Fig. 4) for all PCB congeners, with the exception of CB-47. In contrast, lower polarity solvents, i.e. *n*-hexane and *n*-nonane resulted in a relatively lower peak area response. Toluene which is of medium polarity is also easily immobilised in the fibre and has low water solubility; therefore, it was selected as the most suitable solvent for extraction.

3.4. Effect of salt addition

The addition of salt to an analytical sample can potentially increase analyte recovery in microextraction procedures [34]. Sodium chloride (NaCl) at a concentration between 5 and 33% (w/v) was therefore added to plasma to evaluate its effect on extraction efficiency. Extraction yield improved

with an increasing salt concentration to a maximum value of 15% (see Fig. 5). The same phenomenon has been previously reported [22], where analyte recovery increases due to a decrease in the solubility of the analyte [35]. In contrast to this process, however, there is the possibility that polar molecules may precipitate due to an electrostatic interaction with salt ions in solution [36], thereby decreasing the transfer of PCB congeners across the HFM. Thus, it is reasonable that an initial increase in analyte extraction efficiency occurred with increasing salt concentration, while a further rise decreased the mass transfer of analyte across the HFM. As such, the salt concentration in the sample was optimized at 15% for sample analysis.

3.5. Effect of stir speed

The LPME set-up was agitated with a magnetic stirrer to facilitate mass transfer of PCB congeners from the plasma sample solution to the extraction solvent inside the HFM. Stir speed was optimized to obtain maximum extraction efficiency between 300 and 900 rpm using a 12 mm magnetic stir bar. Fig. 6 shows the enrichment factors for PCB congeners at different stir speeds. A higher stir speed improved the mass transfer of congeners from sample to solvent. LPME supported by HFM is more stable and can
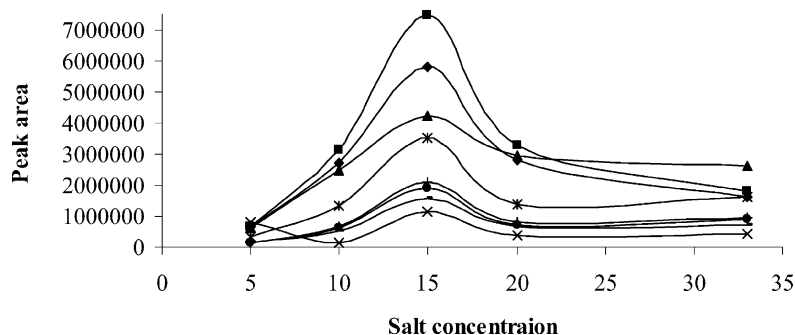

Fig. 5. Extraction efficiency of PCB congeners from blood plasma with added sodium chloride at various concentrations (5–33%): (◆) CB-1; (■) CB-5; (▲) CB-29; (×) CB-47; (✳) CB-98; (●) CB-154; (+) CB-171; (–) CB-200.

Table 2
Liquid-phase microextraction of PCB congeners: enrichment factor, linear range, detection limit and precision (% R.S.D.)

| Congeners	Enrichment factor	R.S.D. (%)[a] $n = 6$	Linearity range (μg/l)	Coefficient of correlation (r)	Limit of detection (μg/l)
CB-1	241	10.2	2.5–150	0.9877	0.07
CB-5	150	8.2	2.5–200	0.9856	0.27
CB-29	183	2.5	2.5–150	0.9917	0.05
CB-47	86	5.0	2.5–200	0.9999	0.44
CB-98	70	4.0	2.5–200	0.9968	0.61
CB-154	67	6.4	2.5–200	0.9969	0.94
CB-171	79	10.8	2.5–200	0.9998	0.82
CB-200	67	4.2	2.5–200	0.9996	0.93

[a] Reproducibility was investigated at a concentration of 25 μg/l of each PCB congener in blood plasma samples.
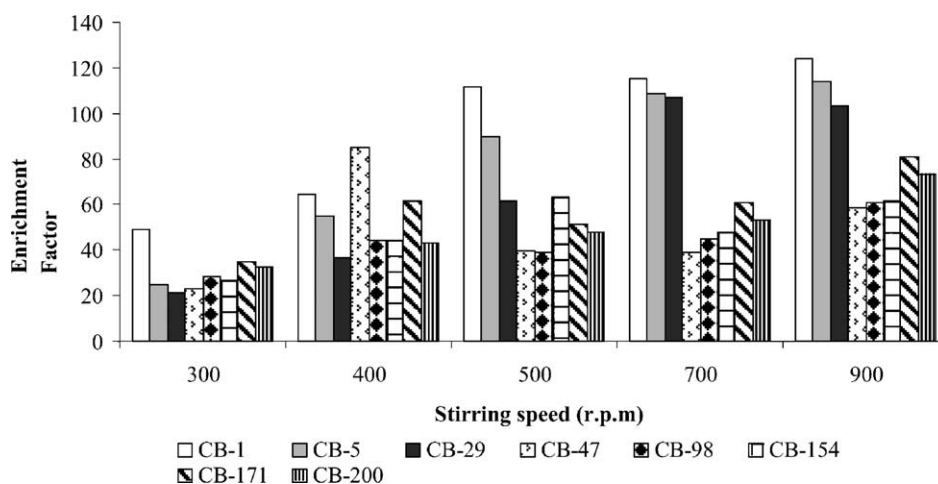
Fig. 6. Effects of sample stir speed on the PCB congener extraction efficiency (blood plasma spiked at 40 μg/l of each congener).

tolerate a higher agitation speed than LPME conducted on a single solvent drop [37,38]. A higher rate of agitation increases the diffusion rate and reduces the time required to reach analyte equilibrium between the sample solution and extraction solvent. Although an agitation rate of 900 rpm resulted in the greatest enrichment factor, air bubbles formed in the HFM due to mechanical forces generated which, in turn, led to occasional difficulties in the quantification of the analytes. Therefore, an optimum stir speed of 700 rpm was selected for sample analysis.

3.6. Extraction pH

As in the LLE of PCBs, sample pH plays important role in LPME [39,40]. As such, a wide range of sample pH from 2 to 13 was evaluated. An acidic range of pH was achieved by adding dilute 6 M hydrochloric acid and a basic pH range by adding phosphate buffer solution. The maximum enrichment factor for the eight PCB congeners was achieved with a sample solution pH of 10.5 (Fig. 7). A pH above or below 10.5 resulted in a decreased extraction efficiency. At this
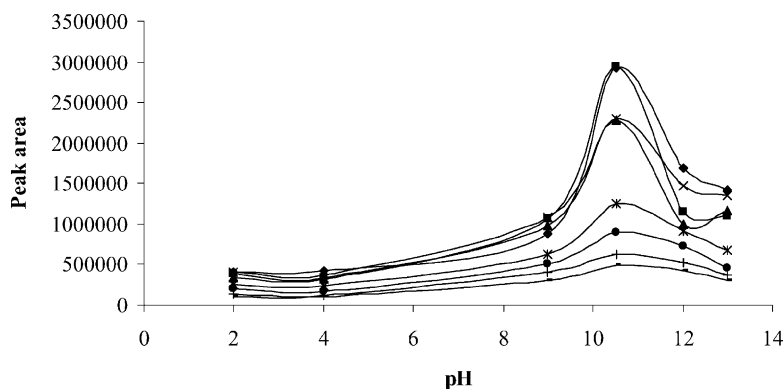

Fig. 7. Effect of sample pH on peak areas of PCBs in spiked blood plasma: (♦) CB-1; (■) CB-5; (▲) CB-29; (×) CB-47; (✕) CB-98; (●) CB-154; (+) CB-171; (–) CB-200.
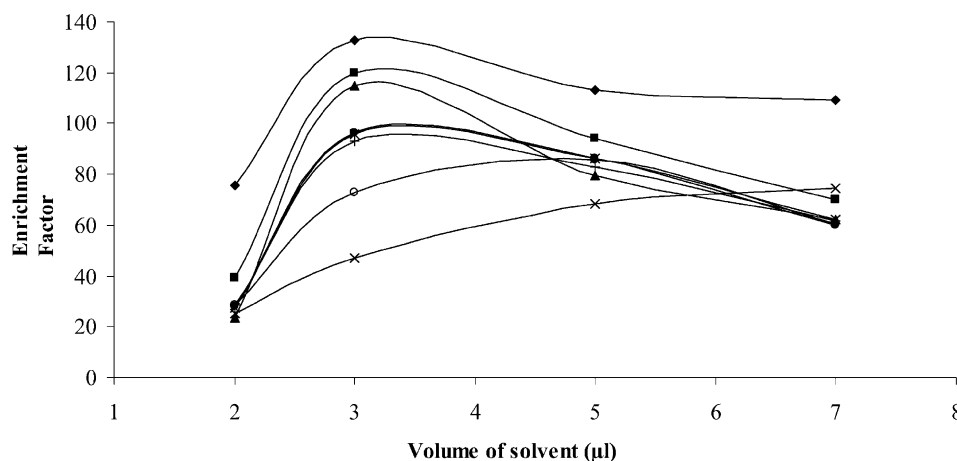
Fig. 8. Effect of toluene volume on extraction efficiency: (◆) CB-1; (■) CB-5; (▲) CB-29; (×) CB-47; (✖) CB-98; (●) CB-154; (+) CB-171; (○) CB-200.

point, it is uncertain why the extraction is pH dependent. It is possible that the optimum pH prevent interfering substances (for example, proteins) in the complex matrix from affecting the extraction. Phosphate buffer (100 μl) was added to the 5 ml sample to achieve pH 10.5 prior to extraction.

3.7. Volume of solvent used

The PCB congeners studied are hydrophobic organic compounds with log values of octanol–water partition coefficients (K_{ow}) ranging from 4.3 to 8.2. As such, these analytes can be expected to readily partition into the water immiscible solvent. The effect of extraction volume, using toluene as the solvent between 2 to 7 μl, was investigated. The enrichment factor of the analyte was dependant on volume used (Fig. 8), where the highest response of PCBs occurred when 3 μl of solvent was used (see Fig. 8). A lower amount (i.e. 2 μl) of solvent was not sufficient to remove the aqueous layer from the microsyringe. Therefore, 3 μl was selected as an extraction volume for sample analysis.

3.8. Sample matrix effect

Extraction of trace analytes from biological samples represents a significant challenge in analytical chemistry due to matrix complexity and interference. The LPME technique provides the necessary selectivity for precision analysis. Fig. 2 shows the chromatograms of extracted blank, spiked and real blood plasma samples. They indicate selective extraction and accurate quantification of PCB congeners using the optimized LPME technique with HFM. However, as blood plasma contains lipids, the extraction efficiency of raw blood plasma samples was dramatically reduced as lipids conceivably block the pores of the HFM, thereby adversely affecting the mass transfer of analyte. To increase the enrichment factor, samples were diluted at a ratio of 1:1 with ultrapure water containing 15% NaCl (w/v) and phosphate buffer (pH 10.5). Dilution gave acceptable congener

recoveries (>76%) and reproducibility (R.S.D. < 9.7%) (Table 3).

3.9. Quantification of PCB congeners in blood plasma samples

In order to evaluate the practical application of the method, congener extraction precision, linearity and the limits of detection (LODs) for PCB congeners were determined. An external calibration curve was constructed using blood plasma samples spiked with the eight PCB congeners at concentrations between 2.5 and 150 μg/l, or 2.5 and 200 μg/l (Table 2). All PCB congeners gave linearity with (coefficient of correlation (r)) values ranging between 0.9877 and 0.9999. Detection limits were calculated by progressing decreasing the analyte concentration in the standard sample by GC–MS-SIM such that signals were clearly discerned at S/N of 3 at the final lowest concentration. Table 2 lists the LODs obtained using LPME with HFM for each of the eight congeners studied. The R.S.D. for sample spiked with 25 μg/l (six replicates) were less than 3% for CB-29, 5% for CB-47, 4% for CB-98 and less than 11% for CB-171 (see Table 2). To obtain acceptable R.S.D. values, optimized conditions were strictly maintained for sample extraction. The external calibration was linear and PCB

Table 3
PCB congener recoveries from blood plasma spiked at 40 and 100 μg/l

Congeners	Relative recovery of plasma sample (%)[a]			
	40 μg/l	R.S.D. (%)	100 μg/l	R.S.D. (%)
CB-1	79.72	8.7	98.00	9.7
CB-5	76.11	8.3	105.46	7.3
CB-29	92.49	8.3	81.69	2.5
CB-47	106.92	8.8	102.82	2.7
CB-98	100.18	9.1	105.50	3.4
CB-154	104.47	8.4	99.19	1.3
CB-171	105.73	8.6	90.35	2.4
CB-200	103.47	8.4	98.50	4.2

[a] With (1:1) dilution, $n = 3$.

Table 4
Concentrations of PCB congeners in blood plasma samples from some hospital patients

Congeners	Concentrations in µg/l (standard error)		
	Indian ($n = 6$)	Malay ($n = 6$)	Chinese ($n = 6$)
CB-1	0.25 ± 0.19	0.19 ± 0.01	0.06 ± 0.01
CB-5	12.54 ± 1.65	0.25 ± 0.28	0.37 ± 1.66
CB-29	5.91 ± 4.28	0.77 ± 0.01	7.89 ± 3.50
CB-47	11.66 ± 2.60	4.92 ± 0.03	21.27 ± 3.50
CB-98	0.18 ± 0.04	0.52 ± 0.01	0.56 ± 0.04
CB-154	2.70 ± 0.76	1.26 ± 0.01	1.84 ± 0.02
CB-171	7.07 ± 1.24	0.60 ± 0.02	1.64 ± 0.06
CB-200	6.60 ± 1.46	1.00 ± 0.03	2.12 ± 0.04
Σ	46.91	9.51	35.75

recoveries and R.S.D. were calculated on the basis of three extractions for two different PCB congener concentrations, i.e. 40 and 100 µg/l. LPME is a non-exhaustive procedure; therefore, relative (rather than absolute) recoveries were calculated and they ranged from 76 to 107% (see Table 3) with R.S.D. values between 1.3 and 9.7%. These results further demonstrated that the LPME–HFM–GC–MS system is highly effective for analysing trace PCBs in blood plasma samples.

3.10. Blood sample analysis

The LPME technique was applied to isolate the PCB congeners from collected blood samples. Prior to extraction, samples were diluted at a ratio on 1:1 with phosphate buffer (pH 10.5), 15% (w/v) NaCl and spiked with the PCB mixture using the procedure previously described. The total ion chromatogram of a real contaminated blood plasma sample is shown in Fig. 2c. The highest mean total concentration of PCBs was detected in (real) samples from the six Indian subjects (i.e. 46.91 µg/l) and the lowest concentration from the Malay subjects (i.e. 9.51 µg/l). The mean total concentration from the Chinese subjects was 35.75 µg/l (Table 4). It is not clear at this stage why there appears to be race-based differences in blood plasma PCB concentrations. The sample size is not large enough for us to speculate on these differences at this time, and in any case detailed discussion of this issue is beyond the scope of the present study. Taking the 18 subjects together, the mean total PCB concentration was 5.12 µg/l. This value may be compared to that measured in the blood of US residents (i.e. 6 µg/l) [9,10], and in infants in Taiwan (i.e. mean of 49.3 µg/l; maximum, 456 µg/l) [41,42] (it should be mentioned that in the US and Taiwanese studies, only total PCB concentrations were reported. No qualitative or quantitative information on specific congeners was provided).

4. Conclusions

A new method for accurately quantifying a range of PCB congeners in complex, "dirty" sample such as human blood plasma using an optimized LPME technique in conjunction with HFM has been successfully developed. The technique has distinct advantages over conventional methods with respect to extraction time and volume of solvents required, where a high level of precision and low detection limits are readily achievable. Sample preparation using the optimized technique is highly efficient and the technique is a selective tool for the separation and enrichment of individual PCB congeners from the blood plasma sample. This method could be effectively applied to clinical toxicological studies.

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