Journal of Chromatography A 1637 (2021) 461828

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Enhanced microextraction of endocrine disrupting chemicals adsorbed on airborne fine particulate matter with gas chromatography–tandem mass spectrometric analysis

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ARTICLE INFO

Article history:
Received 25 September 2020
Revised 1 December 2020
Accepted 17 December 2020
Available online 19 December 2020

Keywords:
Dispersive-liquid-liquid microextraction
Micro-solid-phase extraction
Endocrine disrupting chemical
Airborne fine particulate matter
Gas chromatography–tandem mass spectrometry

ABSTRACT

A novel double-microextraction approach, combining dispersive liquid-liquid microextraction (DLLME) and vortex-assisted micro-solid-phase extraction (VA-µ-SPE) was developed. The procedure was applied to extract endocrine disrupting chemicals (EDCs) consisting of three phthalate esters (PEs) and bisphenol A (BPA) associated with $PM_{2.5}$ (airborne particulate matter with aerodynamic diameter $\leq$ 2.5 µm). Gas chromatography–tandem mass spectrometry (GC–MS/MS) was used for determination of the analytes. These analytes were first ultrasonically desorbed from $PM_{2.5}$ in a 10% acetone aqueous solution. DLLME was used to first preconcentrate the analytes; the sample solution, still in the same vial, was then subjected to VA-µ-SPE. The synergistic effects provided by the combination of the microextraction techniques provided advantages such as high enrichment factors and good cleanup performance. Various extraction parameters such as type and volume of extractant solvent (for DLLME), and type of sorbent, extraction time, desorption solvent, volume of desorption solvent and desorption time (for µ-SPE) were evaluated. Multi-walled carbon nanotubes were found to be the most suitable sorbent. This procedure achieved good precision with intra- and inter-day relative standard deviations of between 1.93 and 9.95%. Good linearity ranges (0.3-100 ng/mL and 0.5-100 ng/mL, depending on analytes), and limits of detection (LODs) of between 0.07 and 0.15 ng/mL were obtained. The method was used to determine the levels of PEs and BPA in ambient air, with concentrations ranging between below the limits of quantification and 0.48 ng/m^3. DLLME–VA-µ-SPE-GC–MS/MS was demonstrated to be suitable for the determination of these EDCs present in $PM_{2.5}$.

1. Introduction

Atmospheric particulate matter (PM) comprises an assortment of solid and aqueous species, emitted via anthropogenic and natural routes, with a range of morphological, physical and chemical properties. The consequences of PM discharging into the atmosphere includes poor air quality, reduced visibility, adverse climate change, and harmful impacts on human health and natural ecosystems [1-3]. PM comprises particles with aerodynamic diameters of $\leq$ 10 µm (PM_{10}), and those of $\leq$ 5 µm ($PM_{2.5}$). The respirable fractions, (i) between $PM_{2.5}$ and PM_{10}, and (ii) below $PM_{2.5}$, are considered the most hazardous to human health. The regulatory concern today is thus focused on these particles small enough to breach the human thoracic region [4].

Endocrine disrupting chemicals (EDCs) such as phthalate esters (PEs), bisphenol A (BPA) and polychlorinated biphenyls, have been extensively studied in relation to their presence in the environment. EDCs have adverse effects on animals and humans, including, as their name indicates, interfering with reproductive and immune systems [5-7]. EDCs may be toxic to the humans when inhaled directly even at trace levels. It has been reported that these compounds are one of the major components of organic aerosols [8]. Among EDCs, PEs and BPA are two main categories which are ubiquitous in the environment like water, soil, sediment, air and food [9,10], as they are extensively involved in the daily lives of human beings [11]. PEs are widely used as plasticizers for polymeric materials for improving flexibility and workability whereas BPA are found in epoxy resins, polycarbonates, and other polymers. Vari-

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https://doi.org/10.1016/j.chroma.2020.461828

ous studies have confirmed that these organics can cause male reproductive disorders and medical problems such as infertility, decreased sperm counts and prostate cancer, etc. [12-14].

The concentrations of the EDCs in the atmosphere are very low. Thus, sample preparation plays a crucial role as part of an analytical methodology to determine trace levels of EDCs in atmospheric PM. Solvent extraction is usually used to extract organic compounds associated with PM collected on filters by vacuum. The most common contemporary extraction methods are ultrasonic-assisted extraction (UAE) [15], microwave-assisted extraction (MAE) [16] and pressurized liquid extraction (PLE) [17]. This is normally followed additional cleanup steps (if needed) before chromatographic separation and analysis with a variety of detection approaches.

There have been several recent studies of EDCs in atmospheric PM. Li and Wang reported the compositions, size distributions and seasonal variations of EDCs in two mega-cities in China [7]. The extraction of $PM_{2.5}$ and PM_{10} collected on quartz microfiber filters was carried out by ultrasonication with a mixture of dichloromethane (DCM) and methanol (MeOH). Subsequently, evaporation, elution and derivatization were conducted before the analytes were determined by gas chromatography–mass spectrometry (GC–MS). In another study, eight PEs associated with PM_{10} and $PM_{2.5}$ were collected in a heavily populated area of Mexico City, Mexico [18]. The PEs were extracted by dry ultrasonication which dislodged the particles mechanically, followed by elution using toluene for determination with GC–tandem MS (MS/MS). An assessment of lung exposure to these contaminants was made. Seasonal variations of the total amounts of eight PEs in $PM_{2.5}$ collected in Shenzhen, China, in dry and wet seasons were studied by Lu et al. [19]. $PM_{2.5}$-entrained quartz fiber filters were ultrasonicated in a mixture of hexane and acetone to extract the PEs. After centrifugation, drying under nitrogen gas and reconstitution with hexane, extracts were analysed by GC–MS. In Northwest Spain, thirteen $PM_{2.5}$-bound estrogenic EDCs were determined in industrial, urban and non-polluted suburban areas [20]. The $PM_{2.5}$ was collected on quartz microfiber filters and extracted by PLE over three cycles using MeOH (100 %) as extraction solvent. The extract was concentrated in a nitrogen gas flow, then dried in an evaporator, and reconstituted with MeOH. Determination was carried out using LC–MS/MS.

Miniaturized sample preparation procedures have attracted immense attention since the early 1990s due to their efficiency, ease of handling and operation, use of much lower quantities of samples, solvents, reagents and sorbents, and generation of minimal waste [21]. Liquid-phase microextraction (LPME) is an example of a miniaturized sample preparation technique. It is now considered to be a generic term for a multitude of equilibrium-based microscale solvent extraction procedures characterized by their use of microliter volumes of solvents. Dispersive-liquid-liquid microextraction (DLLME) is a mode of LPME [22] in which a small volume of an organic extractant solvent is dispersed in an aqueous sample to extract target analytes and is then isolated for analysis. In general, a dispersive solvent is often used in DLLME to facilitate this dispersion that is manifested by the formation of a cloudy solution. This increases enormously the surface area between the organic droplets and the bulk aqueous solution, facilitating extraction. Whether a dispersive solvent is needed is dependent on the extractant used. For example, for 1-octanol, a dispersive solvent is not required since effective dispersion of droplets can be enabled by vortex agitation [23].

An example of a miniaturized sorption-based extraction approach is membrane-protected micro-solid-phase extraction (μ-SPE). In this procedure, a polypropylene (PP) membrane bag housing a sorbent is simply placed in an aqueous sample solution to adsorb the analytes [24]. The PP bag protects the sorbent from the interfering effects of a complex liquid matrix. The major advantage enjoyed by μ-SPE is that cleanup and preconcentration take place concurrently.

Successful applications of μ-SPE in $PM_{2.5}$ research have been reported. One example is a study of MAE-μ-SPE with high-performance liquid chromatography–ultraviolet detection (HPLC–UV) which was applied to quantify the levels of EDCs in total suspended PM [1]. The μ-SPE sorbent was copper II isonicotinate. Another μ-SPE study reported the extraction of glyoxal and methylglyoxal with on-sorbent derivatization by 2,4-diphenylhydrazine [25].

One of the first studies of combined DLLME and μ-SPE (dispersive mode) was reported by Shi and Lee [23]. The appeal of the combined DLLME-μ-SPE approach is that there is no necessity of extract retrieval in the first step, which high enrichment capability is taken advantage. The membrane device is then directly added to the same vial for μ-SPE. This combined microextraction technique, with GC-MS/MS, were applied to the determination of PAHs in $PM_{2.5}$ [26]. On the other hand, Guo and Lee reversed the order of the procedures (i.e., μ-SPE-DLLME) to extract and enrich PEs in water [27]. Finally, Majedi and Lee also applied a combined extraction method for aliphatic amines associated with $PM_{2.5}$ [28]; however, the second step after the DLLME part was classical dispersive SPE, not μ-SPE.

When the aforementioned microextraction procedures are applied to PM, it should be noted that the particulates have to be first taken into solution of an organic solvent-aqueous mixture to desorb the associated contaminants [25,26]. This approach is less harsh than MAE or PLE, another attractive feature of the use of microextraction for PM sample processing.

In this work, a method consisting of combined DLLME-VA-μ-SPE and GC-MS/MS was developed to determine the levels of four EDCs (three PEs and BPA) in atmospheric $PM_{2.5}$ for the first time. Prior to the extraction and preconcentration of the analytes by DLLME-VA-μ-SPE, the $PM_{2.5}$ was rendered into solution form by ultrasonication with an organic-aqueous solvent. The method was applied to the analysis of the PEs and BPA in genuine atmospheric $PM_{2.5}$.

2. Experimental

2.1. Chemicals and materials

Pure diethyl phthalate (DEPH), dibutyl phthalate (DBPH), di (2-ethylhexyl) phthalate (DEHPH), BPA and 1-octanol were obtained from Sigma Aldrich (St. Louis, MO, USA). Graphene oxide (GO), reduced graphene oxide (r-GO) and activated carbon (AC) were supplied by Daejung Chemicals and Metals Co., Ltd (Gyeonggi-do, Shiheung-city, Korea) and multi-walled carbon nanotubes (MWCNTs) (10–20 nm in diameter, specific surface area 90–120 m^2 g^{-1}) were obtained from Shenzhen Nanotech Port Co., Ltd. (Guangdong, Shenzhen, China). HPLC-grade acetone, acetonitrile (ACN), MeOH, DCM, ethyl acetate (EA) and chloroform were purchased from Thermo Fischer Scientific (Waltham, MA, USA). Ultrapure water was prepared on an Evoqua Ultra Clear purification system (Evoqua Water Technologies, Pittsburgh, PA, USA). Q3/2 Accurel 2E HF (RP) PP sheet membrane (~157-μm thickness, 0.2 μm pore size) was supplied by 3M Deutschland (Wuppertal, Germany).

Individual stock solutions of PEs and BPA were prepared in ACN at 2000 mg/L. Mixed standard solutions were prepared at 100 mg/L and 1 mg/L concentrations from the stock solutions by dilution with ACN. All stock solutions and calibration standards were stored in the dark and kept at 4°C. Subsequent working solutions used for the optimisation procedure were prepared in ultrapure water from 1 mg/L mixed standard solution. The working solutions were adjusted to pH 5 with 1M hydrochloric acid or sodium hydroxide solution.

Table 1
Retention times, MRM transitions and collision energy of analytes

Analyte	Retention time (min)	MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)
DEPH	5.98	149.0>65.1	21	149.0>93.1	15	149.0>121.1	9
DBPH	8.96	149.0>65.1	21	149.0>93.2	15	149.0>121.1	12
BPA	11.54	213.0>119.1	12	213.0>91.1	27	119.0>91.1	9
DEHPH	15.37	167.0>149.0	9	149.0>65.1	24	149.0>93.0	18

2.2. Instrumentation

Analysis was performed using Shimadzu (Kyoto, Japan) Model 8040 GC–triple quadrupole MS/MS system equipped with a Shimadzu AOC-20i autosampler and DB-5MS (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m x 0.025 mm (i.d), 0.025 μm film thickness). Helium (purity 99.9999%) was employed as the carrier gas at a flow rate of 1.3 mL/min. Samples were injected into the GC under splitless mode. The injector and interface temperature were set at 230°C. The GC oven temperature was programmed as follows: Initial temperature of 105°C for 1 min, increased to 200°C at 20°C/min and held for 1 min, followed by an increase to 220°C at 10°C /min and held for 2 min, and then increased to 300°C at 10°C/min and held for 2 min. The temperature of the electron impact ion source, operated at 70 eV, was set at 230°C. The standard solutions and real sample extract were analysed in multiple reaction monitoring (MRM) mode. The masses monitored by the detector were set as follows: DEPH, mass-to-charge ratio *m/z* 65, 149; DBPH, *m/z* 65, 149; BPA, *m/z* 119, 213; and DEHPH, *m/z* 149, 167. All GC–MS/MS analyses were performed in triplicate. The retention times, MRM transitions and collision energies of the analytes are shown in Table 1.

2.3. Extraction of analytes

2.3.1. Preparation of µ-SPE device

The µ-SPE device was prepared, following a procedure previously reported by Basheer et al. [24]. In brief, a piece of PP membrane (ca. 2.5 cm x 2 cm) was folded in half along its longer length, and heat-sealed along two of the edges to create an envelope or bag with an opening. Three milligrams of sorbent were loaded into the bag via the opening that was then heat-sealed to secure the contents [29]. The final dimension of the PP bag was ca. 1.5 cm x 1 cm. The µ-SPE device was conditioned by ultrasonication in acetone for 10 min and stored in the same solvent until use.

2.3.2. Processing of $PM_{2.5}$-entrained filters

A polytetrafluoroethylene (PTFE)-coated glass filter (GP 47 P; 47mm diameter) (Pall Corporation, Ann Arbor, MI, USA) was first treated with 5% nitric acid, then with MeOH/tetrahydrofuran (1:1) and subsequently n-hexane for cleaning prior to collection of $PM_{2.5}$. The PM was collected through a cyclone air sampler (URG-2000-30FG, University Research Glass, Chapel Hill, NC, USA) at a flowrate of 16.7 L/min for 24 h in Oct 2018 at the National University of Singapore (total volume of air sampled: 24.048 m^3). Afterwards, the filter was wrapped in an aluminium foil and stored at 4°C until further use.

A filter on which genuine atmospheric $PM_{2.5}$ was collected was divided into quarters. One quarter-piece was processed by placing it into a beaker containing 30 mL of an aqueous solution containing 10% acetone. The contents were ultrasonicated for 15 min, after which the sample solution was used for DLLME-VA-µ-SPE (Section 2.3.3), which was also applied to a similarly treated blank filter to quantify its background characteristics, and to determine the analytical figures of merit (i.e., limits of detection (LODs), limits of quantification (LOQs), etc). Blank and sampled filters onto which the analytes were directly spiked at 1 ng/mL and 5 ng/mL were also processed by DLLME-VA-µ-SPE similarly to determine relative recoveries (%RRs). Filtration or any other pre-treatment was not applied to the genuine filter sample solutions to prevent possible loss of analytes.

2.3.3. DLLME-VA-µ-SPE

For each extraction experiment, 8 mL of aqueous solution (pre-adjusted to pH 5) spiked with standard analytes to give a final concentration of 50 ng/mL of each EDC, was added into a 10 mL clear glass vial. In the DLLME step, a mixture of 400 µL of acetone (dispersive solvent) and 70 µL of chloroform (extractant solvent) was rapidly injected into the solution using a syringe. This was to achieve dispersion (indicated by a cloudy solution consisting of tiny droplets) to create a large surface area for efficient extraction of analytes in the aqueous solution). After 2 min, the µ-SPE device was added to the solution, and the contents were vortexed after the vial was capped, for 10 min of µ-SPE. Here, the EDCs were first extracted into chloroform (DLLME), and then adsorbed on the MWCNTs (µ-SPE). Upon completion of extraction, the µ-SPE device was retrieved with a pair of tweezers, washed with ultrapure water and dabbed dry using lint-free tissue paper. It was then placed into a microcentrifuge tube for solvent desorption in 100 µL acetone by ultrasonication for 10 min. One microlitre of the extract was injected into the GC–MS/MS system for analysis. After desorption, the used µ-SPE device was regenerated by ultrasonication with MeOH, and then acetone separately. Fig. 1 shows the schematic of the DLLME–VA-µ-SPE procedure.

The same combined extraction procedure was also applied to the solutions obtained from the processing of the blank filter, and genuine $PM_{2.5}$-entrained filters. Most of the microextraction procedures are equilibrium-based with their use of small volumes of extractant solvent relative to the volumes of the sample solution [30]. To determine the effectiveness of microextraction techniques, two parameters, %RR and enrichment factors (EFs), are usually calculated.

The %RR can be measured using the following relationship:

$$\%RR = [(C_{ss} - C_s)/C_{sw}] \text{ x } 100$$

where C_{ss}, C_s and C_{sw} refer to the analyte concentrations found in the sample after spiking with standards, the analyte concentrations determined in the genuine sample, and the analyte concentrations in a spiked aqueous standard solution, respectively.

The EF enabled by an extraction method with respect to an analyte can be calculated using

$$EF = C_f / C_i$$

where C_f is the final concentrations of an analyte in the organic extractant, and C_i the initial concentration of the analyte in the standard sample solution.

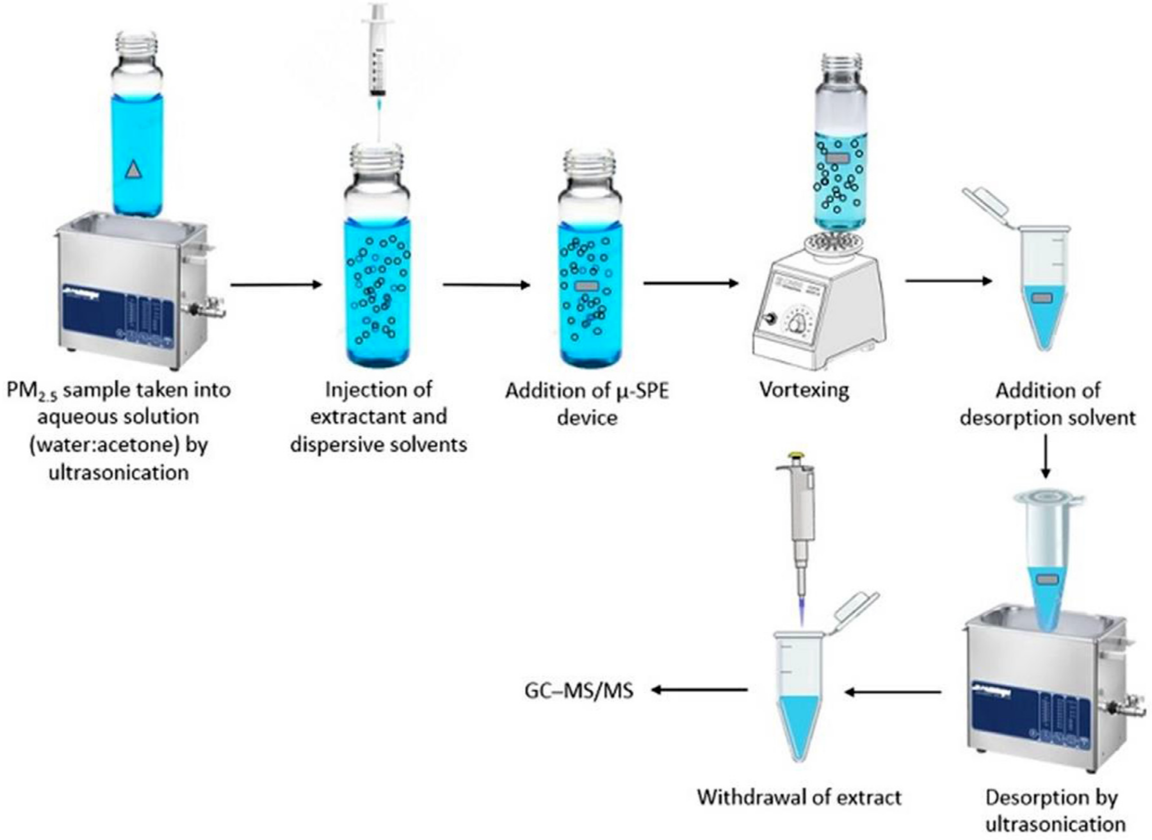

Fig. 1. Schematic of DLLME–VA-µ-SPE–GC–MS/MS.

3. Results and discussion

3.1. Evaluation of extraction conditions

3.1.1. Type of extractant solvent

The extractant solvent is one of the most important parameters to be optimised in the DLLME procedure. The criteria for a preferred extractant solvent include: low water miscibility and low viscosity to form an emulsion effectively, good extraction capability of target analytes, good chromatographic performance, and compatibility with the µ-SPE membrane material, it is able to diffuse expeditiously through the pores to adsorb on the sorbent [31] .

Fig. 2. shows that chloroform is the most favorable extractant solvent for all target analytes. As this solvent is hydrophobic and halogenated [32], it has good affinity with the target analytes through hydrophobic interactions with respect to the 'like dissolves like' principle. It was also observed that chloroform was the only solvent that could effectively extract BPA. This may be due to the higher solubility of BPA in chloroform, while the relatively inferior result for DCM was conceivably due to the lower solubility in this solvent [33].

The non-chlorinated solvents such as 1-octanol and EA were the poorest extractant solvents, probably caused by the inefficient formation of emulsion during the DLLME step of the extraction process due to their high viscosity and high aqueous solubility, respectively [31,34,35].

Although use of chloroform is usually not recommended due to its toxic properties, in the present case, its DLLME performance was far superior to that of the other solvents. The necessity of developing a highly efficient extraction procedure took precedence, especially since only microliter volumes were needed.

3.1.2. Volume of extractant solvent

The volume of extractant solvent is crucial in determining the DLLME efficiency as the EF depends on the volume ratio of the sample solution to the extraction phase [36]. Although a low extraction solvent volume contributes to a higher EF, its capacity for extracting the analytes needs to be considered [30]. A range of solvent volumes (between 50 µL and 150 µL) was evaluated (Fig. 3). It can be seen that 70 µL was the most favorable volume that provided the highest overall extraction efficiencies. Lower extraction efficiencies at volumes beyond 70 µL were most likely due to the dilution effect which predominated over extraction capacity [37].

3.1.3. Selection of sorbent

A comparison of the extraction efficiencies provided by four commercial sorbents, MWCNTs, GO, rGO and AC, for µ-SPE was carried out. The results (Fig. 4) showed that MWCNTs were the most effective sorbent in extracting the target analytes since MWCNTs interact favorably with organic molecules containing aromatic rings through π interactions [25]. Studies have shown that the material has excellent adsorption or extraction capability for both BPA-type EDCs and PEs [38,27]. The adsorption mechanisms in respect of both PEs and BPA on MWCNTs are due to compatibility of hydrophobic character and π interaction between the aromatic rings, with, for BPA, additional hydrogen bonding formation [38,39].

In contrast, GO and rGO exhibited lower extraction efficiencies than MWCNTs. Both materials displayed similar extraction capability. A possible reason for this observation is that whereas GO can form stronger hydrogen bonding with PEs and BPA due to its larger number of carbonyl and hydroxyl functional groups [40], rGO has stronger hydrophobic interactions with these analytes [40-42]. The overall effect was that both had comparable observable extraction performance. Lastly, granular AC, the pure carbon-based sorbent,

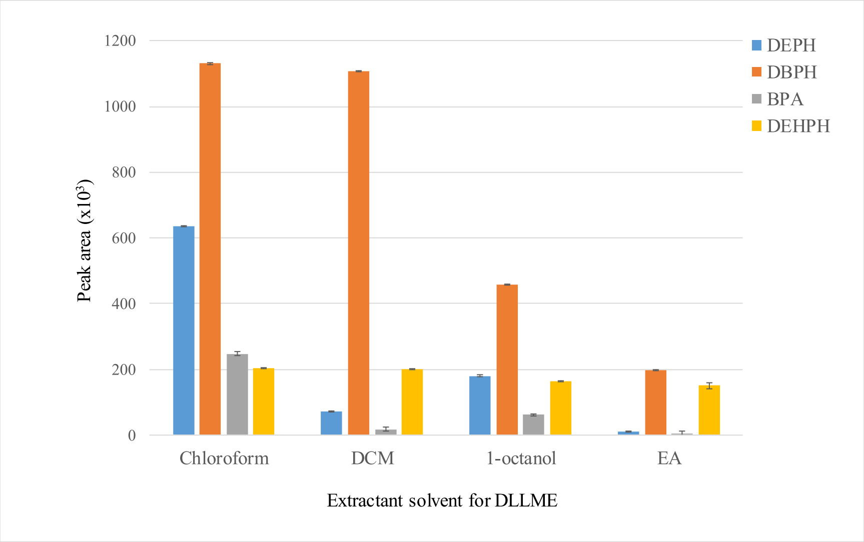

Fig. 2. Effects of extractant solvent on DLLME–VA-μ-SPE–GC–MS/MS. Analyte concentrations: 50 ng/mL of each; solvent volume: 100 μL; DLLME extraction time: 2 min; sorbent: MWCNTs; μ-SPE vortex (extraction) time: 10 min; desorption solvent: acetone; volume of desorption solvent: 100 μL; desorption (sonication) time: 10 min.

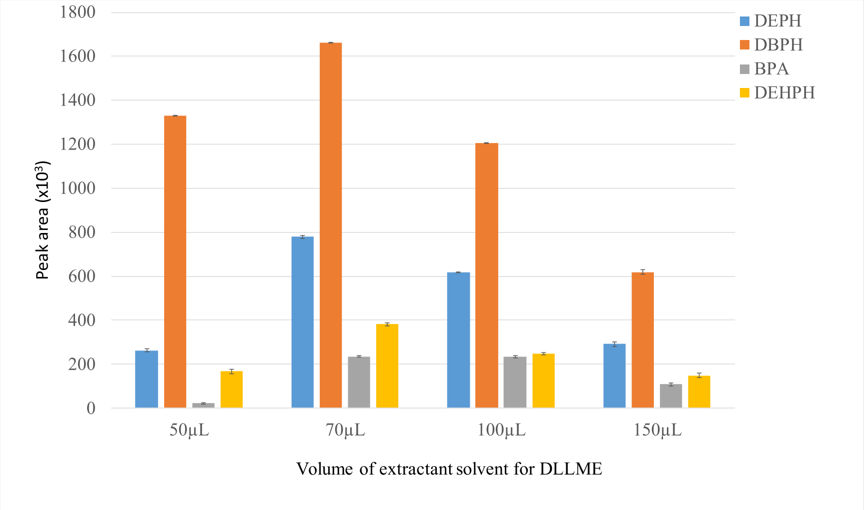

Fig. 3. Effect of volume of extractant solvent on DLLME–VA-μ-SPE–GC–MS/MS. Analyte concentrations: 50 ng/mL of each; extractant solvent: chloroform; DLLME extraction time: 2 min; sorbent: MWCNTs; μ-SPE vortex (extraction) time: 10 min; desorption solvent: acetone; volume of desorption solvent: 100 μL; desorption (sonication) time: 10 min.

is hydrophobic. AC preferentially binds to substances of low polarity with high molecular weights due to its large pore size and void volume [25], resulting in this material having the lowest extraction capability.

The hydrophobicity of MWCNTs and their ability to participate in π interactions as such, coupled with the high surface area they could present to the analytes, allowed the effective enrichment of the analytes. Thus, it was surmised that they were the most suitable sorbent for PEs.

3.1.4. Extraction time

Since the mass transfer of the analytes from the spiked aqueous solution to the μ-SPE device is a time-dependent process, the efficiency of extraction time (from 5 – 15 min) was evaluated. The results indicated that 10 min was the most suitable time for the maximum adsorption of the analytes into the μ-SPE device (Fig. 5). As μ-SPE is an equilibrium-based process, prolonged extraction time could promote reverse partitioning of the analytes back into the solution [31,43], as indicated by the decrease in extraction efficiency over an extraction time of 15 min. It is noted that the extraction time of VA-μ-SPE was significantly shorter than conventional μ-SPE, which uses magnetic stirring for agitation that usually required more than 30 min to extract similar analytes [25,44]. It is clear that vortexing coupled with DLLME, can speed up the extraction process as compared to conventional μ-SPE; this obviously has the advantage of increasing the sample throughput [25,31]. From the experiments, 10 min was thus chosen as the most favorable extraction time.

3.1.5. Desorption solvent

After extraction, the analytes adsorbed on the μ-SPE sorbent were desorbed by an organic solvent under ultrasonication. Several organic solvents were investigated for their desorption capability. The results indicated that the highest desorption efficiency was achieved using acetone (Fig. S1). This is due to the polarity of the solvent which largely determines the solubility of analytes [45]. Since acetone is the least polar solvent (partition coefficient, log P = -0.24), it can most effectively disrupt the interactions be-

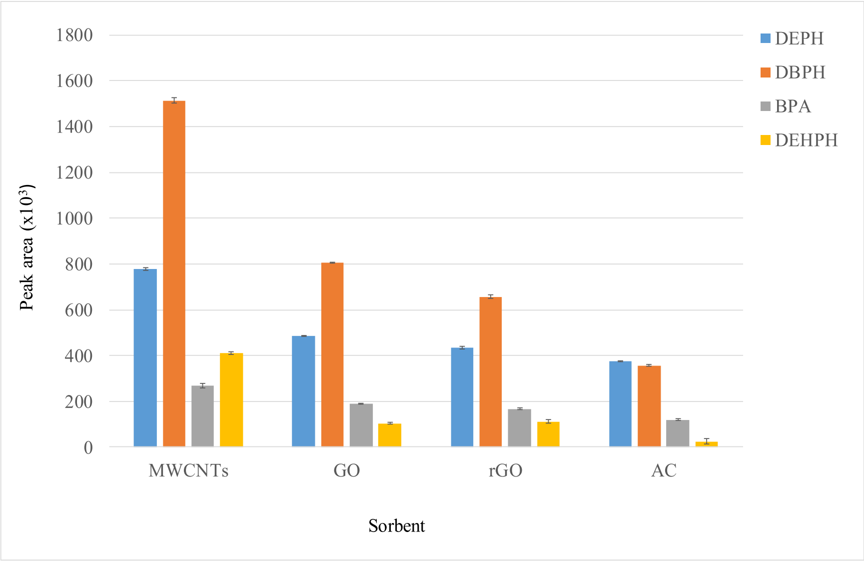

Fig. 4. Selection of sorbent on DLLME–VA-μ-SPE–GC–MS/MS. Analyte concentrations: 50 ng/mL of each; extractant solvent: chloroform; volume of extractant solvent: 70 μL: DLLME extraction time: 2 min; μ-SPE vortex (extraction) time: 10 min; desorption solvent: acetone; volume of desorption solvent: 100 μL; desorption (sonication) time: 10 min.

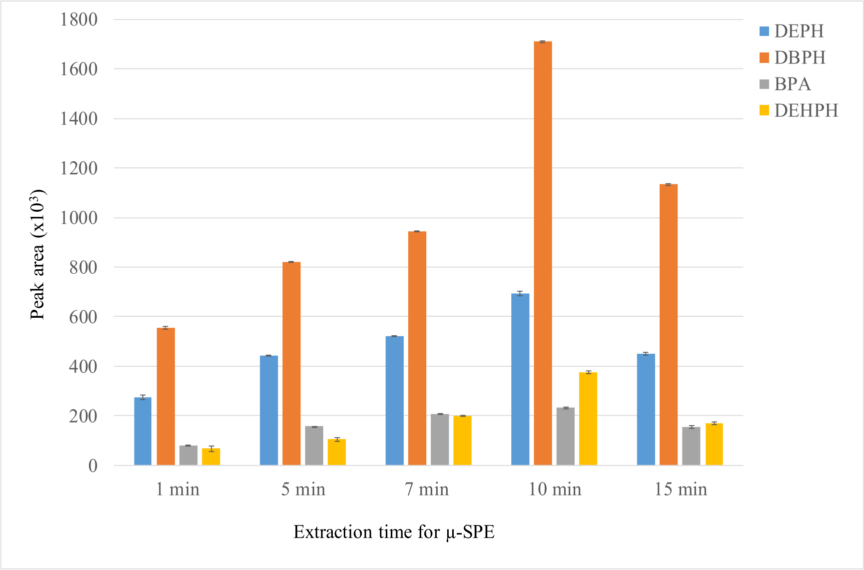

Fig. 5. Effect of extraction (vortexing) time on DLLME–VA-μ-SPE–GC–MS/MS. Analyte concentrations: 50 ng/mL of each; extractant solvent: chloroform; sorbent: MWCNTs; volume of extractant solvent: 70 μL: DLLME extraction time: 2 min; desorption solvent: acetone; volume of desorption solvent: 100 μL; desorption (sonication) time: 10 min.

tween the slightly polar analytes and MWCNTs and can hence displace the analytes from MWCNTs [31,46].

In comparison, MeOH which is the most polar solvent (log P = -0.77) exhibited the lowest desorption efficiency. Mid-polar solvents such as ACN and ethanol (log P = -0.34 and -0.31, respectively) have similar polarities and therefore displayed comparable desorption results. Acetone was thus chosen as the most suitable desorption solvent.

3.1.6. Volume of desorption solvent

In order to elute the target analytes from the μ-SPE device completely and reproducibly, sufficient desorption solvent was required. However, this volume should be minimized to avoid dilution of the analytes that would compromise the overall extraction efficiencies provided by DLLME–VA-μ-SPE. A range of desorption volumes (100, 150, 200 and 250 μL) were used to determine the most favorable solvent volume.

The results indicated that 100 μL provides the maximum chromatographic response of the analytes due to relatively high enrichment of analytes in this solvent volume (Fig. S2). The extraction efficiency decreases significantly beyond 100 μL, due to dilution caused by the use of larger volumes of acetone. One hundred microliters were therefore adopted as the most favourable desorption volume.

3.1.7. Desorption time

The effect of desorption time was examined over the range 3-15 min. Similar to extraction, desorption is also an equilibrium-based process [31]. The results showed that desorption time of 10 min was sufficient to establish an equilibrium between the sorbent and desorption solvent, providing the highest peak areas of the analytes (Fig. S3). Prolonged sonication of > 15 min caused a slight decrease in desorption efficiency due to re-adsorption, which is a common observation reported in other μ-SPE studies [31,47].

Table 2
Analytical figures of merit of the procedure DLLME–VA-μ-SPE-GC-MS/MS

Analyte	Linearity range (ng/mL)	Coefficient of determination (r^2)	LOD (ng/mL)	LOQ (ng/mL)	%RSD (n=3)		EF[a]
					Intra-day	Inter-day	
DEPH	0.3–100	0.998	0.07	0.22	4.66	2.39	23
DBPH	0.5–100	0.998	0.13	0.44	6.40	1.93	86
BPA	0.3–100	0.998	0.08	0.25	8.74	8.75	55
DEHPH	0.5–100	0.995	0.15	0.50	9.95	6.45	62

[a] Calculated based on 0.5 ng/mL of each analyte in ultrapure water sample

Table 3
Concentrations of EDCs in PM2.5 and relative recoveries (RRs)

Analyte	Concentration in $PM_{2.5}$ (ng/m^3)[a]	%RR (n=3)			
		Spiked to concentration of 1 ng/mL	%RSD	Spiked to concentration of 5 ng/mL	%RSD
DEPH	<LOQ	78.2	10.4	73.9	10.7
DBPH	0.27[b]	66.5	6.9	93.0	1.7
BPA	<LOQ	79.1	3.1	61.9	6.2
DEHPH	0.48[c]	70.9	2.8	95.8	8.4

[a] Calculated based on $PM_{2.5}$ filter sampled at the flow rate of 16.7 L/min for 24 h
[b] Value corresponds to 0.21 ng/mL
[c] Value corresponds to 0.38 ng/mL

The reusability of μ-SPE device was evaluated. After one desorption experiment, the μ-SPE device was washed with acetone to remove potential residual analytes, and then reused. No carry-over effects were observed. A decrease in extraction efficiency was recorded only after the same μ-SPE device was used more than 10 times. This may be due to the blockage of the membrane pores after repeated usage of the μ-SPE device [45]. Also, prolonged and continual sonication could have deformed the MWCNT structure over time [48], leading to reduced surface area available for extraction of the analytes. Thus, a single device was reusable up to 10 times without impacting on its extraction performance negatively.

3.2. Comparison of DLLME–VA-μ-SPE, DLLME and VA-μ-SPE

A preliminary comparative evaluation of DLLME–VA-μ-SPE, DLLME and VA-μ-SPE was first carried out using aqueous EDC standard solutions containing 50 ng/mL of each analyte. For μ-SPE, the sorbent used was MWCNTs. As usual, peak areas from the GC-MS/MS analyses were taken to represent the relative performances of the extraction procedures (Fig. S4). The experiments revealed the superior extraction capability of DLLME–VA-μ-SPE, compared to the individual procedures separately, especially towards the less hydrophobic analytes DEPH and BPA. This highlighted the importance of the role DLLME played in enhancing extraction before the droplets containing the analytes diffused into the membrane bag, to be adsorbed by the MWCNTs, μ-SPE sorbent.

3.3. Method validation of DLLME–VA-μ-SPE-GC-MS/MS

Under the most favorable conditions, the analytical performance of the proposed method was assessed by evaluating its calibration linear range, LODs, LOQs, EFs, and intra- and inter-day precision in terms of percent relative standard deviations (%RSDs), as summarised in Table 2.

The linearity range was from 0.3 to 100 ng/mL for DEPH and BPA, and 0.5 to 100 ng/mL for DBPH and DEHPH. The LODs were in the range of between 0.07 and 0.15 ng/mL while the LOQs were from 0.22–0.50 ng/mL. Intra-day repeatability in terms of RSDs was determined to be from 4.66 to 9.95%, whereas inter-day reproducibility was between 1.93 and 8.75%. The EFs, which are defined as the ratios of analyte concentrations in the extracts to those in the samples, were calculated to be between 23 and 86.

3.4. Analysis of $PM_{2.5}$ sample

The developed procedure was subsequently applied to genuine air samples ($PM_{2.5}$-entrained filter) for the quantification of PEs and BPA associated with them. Spiked quarter-portions of sampled filters (at concentrations 1 ng/mL, and 5 ng/mL of each analyte) processed to investigate matrix effects (by measuring %RRs). The calculated %RRs, %RSDs and the levels of the target analytes in ambient air are shown in Table 3. The gas chromatograms of extracts of unspiked, and spiked real samples (at 5 ng/mL of each analyte) are also shown in Fig. S5. The peaks were identified based on their chromatographic retention times and mass spectra ratios. The unknown peak at ca. 8.1 min is possibly an artefact. Since it did not interfere with the analysis, no attempt was made to identify it.

The environmental concentrations of the four EDCs were in the range of <LOQ - 0.48 ng/m^3. The levels of those detected, DBPH and DEHPH, were 0.27 and 0.48 ng/m^3, respectively. This is comparable with the concentration ranges reported for these compounds in airborne PM in other studies [1,19,20] in different regions. The levels of the same EDCs (DEPH, DBPH, DEHPH and BPA) as studied in present work associated with total suspended PM in urban, business centre and industrial sites of Dongguan, China, have been reported to be in the range of N.D.-68.8 ng/m^3 [1], 1.64-59.9 ng/m^3 in $PM_{2.5}$ (for three of the EDCs; DEPH, DBPH and DEHPH) in Shenzhen, China [19], and N.D.-54.7 ng/m^3 in $PM_{2.5}$ (for DEPH, DBPH, DEHPH and BPA) in industrial, urban and suburban areas of A. Coruña (Northwest Spain) [20].

The values of RRs calculated after extraction and analysis of spiked filters were between 61.9 and 95.8%, indicating that there were some matrix interferences involved, which could be attributed to the DLLME part of the procedure. This shows that the use of μ-SPE, where the PP membrane bag acted as a protective barrier, as the second extraction step, was unable to fully overcome the limitation of DLLME in eliminating matrix effects. Nonetheless, μ-SPE still effectively reduced the severity of the matrix effects while taking advantage of DLLME in providing high EFs, there-

Table 4
Comparative data of current and other methods

Analytes[a]	Extraction method[b]	Sample matrix	Detection method	Total volume of solvent (μL); solvent type	Total extraction time (min)	%RR	LOD (ng/mL)	%RSD	Ref.
DEPH, DBPH, BPA, DEHPH	DLLME–VA-μ-SPE	$PM_{2.5}$ extract	GC–MS/MS	170; Chloroform (DLLME), Acetone (μ-SPE)	22	61.9-95.8	0.07-0.15	<10	This work
DEPH, DBPH, BPA, DEHPH	MAE–μ-SPE	Suspended air PM	HPLC–UV	102.5; n-Hexane	40	81.2-119.2	2.0-8.5 ng/L	<10	[1]
DEPH, DBPH, DEHPH	UAE	$PM_{2.5}$ extract	GC–MS	11 mL; n-Hexane: Acetone	40	76.4-110	0.11-0.43 ng/m^3	-	[19]
DEPH, DBPH, BPA, DEHPH	PLE	$PM_{2.5}$ extract	LC–MS/MS	-; MeOH	24	88-121	2.0-12 pg/m^3	<15	[20]
DEPH, DBPH, BPA	EE-SPME	Sea water	GC–MS	-	20	73.9-93.4	0.004-0.15	<5	[48]
DEPH, DBPH, BPA, DEHPH	SB-DLLME	Mineral and tap water	GC–IT/MS	200; n-Hexane	21	93.4-104.5	1-5	<9.3	[49]
DEPH, DBPH, DEHPH	VA-μ-SPE–LDS-DLLME	River water	GC–MS	380; ACN (μ-SPE), n-Hexane (DLLME)	17	89.5-102.3	0.006-0.01	<9.7	[27]

[a] Only analytes identical to those in the present work are listed and compared.
[b] EE: Electro-enhanced; SB: Solvent-based; LDS: Low-density solvent; IT; Ion trap

fore demonstrating the benefit of the double-microextraction technique.

3.5. Comparison with other methods

Table 4 shows comparative data amongst the present and previously reported methods for analysing EDCs in airborne PM. There is no doubt that MAE or PLE, for example, are highly efficient extraction methods for PEs and BPA in suspended airborne PM [1,18,19]. Nevertheless, they are more sophisticated, expensive, and aggressive extraction modes than the present DLLME–VA-μ-SPE technique. Together with GC-MS/MS, DLLME–VA-μ-SPE gave good analytical performance. In this method, the analytes adsorbed on the filter samples were taken into aqueous solution using 3 mL of acetone with ultrasonication. Only 100 μL of the same solvent were needed for analyte desorption. Therefore, overall, there was minimal consumption of organic solvent with generation of little waste in the present method.

DLLME–VA-μ-SPE–GC–MS/MS was also compared with other methods for the analysis of EDCs in various water samples [27,48,49]. The details are also summarized in Table 4. As can be seen, the present technique also compared very well with the other techniques.

4. Conclusion

With the widespread use of plastic products, human exposure to plasticizers and their release into the environment has been inevitable. In particular, PEs and BPA are common plasticizers that are known to cause adverse effects in animals and human beings. Although studies have reported that PM containing these analytes could pose an exposure hazard by inhalation, there has been limited work on the determination of PEs and BPA present in the air. It is thus important to develop analytical methodologies for these analytes present in atmospheric particulate matter that are simple, rapid and provide high efficiency.

In the present work, the combined microextraction technique, DLLME-VA-μ-SPE, was demonstrated to be relatively fast, and has high enrichment factors, good repeatability and reproducibility, and sensitivity for the GC-MS/MS analysis of PEs and BPA in $PM_{2.5}$. In summary, the combined techniques worked synergistically, giving rise to advantages such as high preconcentration of the analytes and good clean-up efficiency, for the GC-MS/MS detection of PEs and BPA present in $PM_{2.5}$ samples.

It is believed that this proposed method can be subsequently expanded to be applied to a wider range of analytes by adopting different solvents and sorbents to tune its selectivity. There is currently a wide variety of sorbents being explored in sorbent-based sample preparation, including μ-SPE, such as polymers, carbon-based materials, zeolites and metal-organic frameworks, all of which are garnering attention in extraction applications.

In addition, future work can include the combinations of other microextraction techniques, or those of microextraction and conventional extraction procedures. As far as μ-SPE is concerned, a current disadvantage is that automation remains challenging. Additional research efforts by our laboratory will be expended in developing a method to fully automate μ-SPE, and hence explore the possibility of automating double-microextraction techniques.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

CRediT authorship contribution statement

Nyi Nyi Naing: Writing - original draft, Writing - review & editing. **Esther Xue Yi Goh:** Investigation, Writing - original draft. **Hian Kee Lee:** Conceptualization, Writing - review & editing.

Acknowledgements

The authors acknowledge the financial support of the National Environment Agency (NEA), Singapore (grant no. R-706-000-043-490), and the National University of Singapore (NUS) (grant no. R-143-000-023-001) for support of this work. We also thank Dr. L.E. Yu of the Department of Civil and Environmental Engineering, NUS, for providing the $PM_{2.5}$ samples. The views of the authors in this article do not reflect those of the NEA.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2020.461828.

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