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Development of natural sorbent based micro-solid-phase extraction for determination of phthalate esters in milk samples

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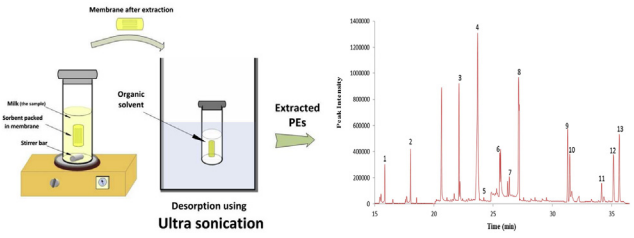
HIGHLIGHTS

- For the first time, seed powder of *Moringa oleifera* was used as sorbent for micro-solid phase extraction.
- A simple and efficient analytical method for quantifying phthalates in milk samples was developed.
- This natural sorbent that is cheap and readily available and its performance was comparable with synthetic sorbents.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, a natural sorbent based micro-solid phase extraction (μ-SPE) was developed for determination of phthalate esters in milk samples. For the first time, an efficient and cost effective natural material (seed powder of *Moringa oleifera*) was employed as sorbent in μ-SPE. The sorbent was found to be naturally enriched with variety of functional groups and having a network of interconnected fibers. This method of extraction integrates different steps such as removal of proteins and fatty stuff, extraction and pre-concentration of target analytes into a single step. Thirteen phthalate esters were selected as target compounds for the development and evaluation of method. Some key parameters affecting the extraction efficiency were optimized, including selection of membrane, selection and amount of sorbent, extraction time, desorption solvent, volume of desorption solvent, desorption time and effect of salt addition. Under the optimum conditions, very good linearity was achieved for all the analytes with coefficient of determinations (R^2) ranging between 0.9768 and 0.9977. The limits of detection ranged from 0.01 to 1.2 μg L^{-1}. Proposed method showed satisfactory reproducibility with relative standard deviations ranging from 3.6% to 10.2% (n = 7). Finally, the developed method was applied to tetra pack and bottled milk samples for the determination of phthalate esters. The performance of natural sorbent based μ-SPE was better or comparable to the methods reported in the literature.

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1. Introduction

Phthalate esters (PEs) are famous class of polymer additives, which are used to introduce plasticity and durability to polyvinyl chloride (PVC) and other plastic materials. Since, they are not chemically bound to the structure of the polymer chains in plastics, they can migrate from plastics to the substances they are in contact with. The considerable environmental concern arises from their migration to food materials and water [1].

PEs have been specially considered from the perspective of their health implications on the human and wildlife. They are reported to induce reproductive, developmental and neurological disorders and thus considered as endocrine disrupters (EDCs) [2–4]. Due to their potential risks to human health and environment, PEs have been placed in priority pollutant list issued by United States Environmental Protection Agency (USEPA). Ingestion of contaminated food is major route of human exposure to PEs. Therefore, it is highly desired to develop sensitive analytical methods for trace level monitoring of PEs in food.

Due to its nutritional value and immunological benefits to infants and aged people, milk stands among imperious and highly consumed foods. Thus, monitoring of potential pollutants such as PEs in milk samples has great significance from human health perspective [5]. Milk can get contaminated with PEs through different sources including environmental diffusion, direct uptake by the animals through air or food, and processing by contact with different plastics, pipes, containers etc. [6]. In dairy forms and milk processing units, bulk transfer of milk between tankers and storage tanks is carried out through plastic pipes which can introduce PEs into milk [7]. Machine milking, where milk comes in contact with rubber parts of the machine, was also reported to enhance PE contents in milk [8].

Since milk represents a very complex matrix which is enriched with fats, lipids, carbohydrates, vitamins and minerals. Extraction of target compounds from milk samples is a rather challenging task. Conventional extraction methods such as liquid–liquid extraction and solid phase extraction require large amounts of solvents, samples and adsorbent materials. Furthermore, they are labor and time extensive and materials have lesser possibility to be reused. Thus, sample preparation scientists are nowadays dedicated to develop efficient, green, inexpensive and miniaturized extraction procedures in order to extract and pre-concentrate the analytes of interest prior to instrumental analysis [5]. Hence, over a period of time, number of microextraction methods have been developed for extraction of PEs in milk samples. In this regard, most commonly used methods are dispersive liquid–liquid extraction (DLLME) [5,9] and headspace solid phase microextraction (HS-SPME) [10]. For DLLME, clean samples are preferred for proper phase separation. Additionally, complex samples like milk cannot be used directly without pre-treatment. In order to remove proteins and fatty stuff from the samples, DLLME involves some additional steps such as use of number of organic solvents, which are not desired when analyzing PEs because PE residues can be found even in organic solvents and they are abundant in laboratory environment [11]. Moreover, pretreatment of proteins and fatty stuff using organic solvents such as methanol and acetonitrile may lead to loss or dilution of the target analytes. HS-SPME also has several limitations when dealing with PEs, particularly most of PEs show low volatility and reasonably high temperatures are required to vaporize PEs. In addition, SPME fibers are expensive and highly fragile [12].

In the current study, we applied porous membrane protected micro-solid phase extraction (μ-SPE) for extraction of PEs in milk samples. This technique was first introduced in 2006 [13] and is increasingly popular sample preparation method for different classes of organic compounds [14–16] present in food [17–19], environmental [20,21], and biological [22,23] matrices. Technique continued to evolve over the period of time and it was assisted by vortex [21], microwave [24], and by combining with other extraction approaches [17,25].

In most of the previous studies, synthetic sorbents were used in μ-SPE. Although these sorbents offer unique advantages such as high affinity and selectivity toward target compounds but their preparation is labor and time extensive due to extended synthesis procedures. Any small variations in synthesis conditions may affect the activity of sorbent to a large extent. Likewise, the consumption of large amount of chemicals for synthesis of selective sorbents is hazardous both for workers and environment and contradicts basic rules of green chemistry approaches. Hence, there is a pressing need to explore green and readily available sorbent materials for extraction applications. Thus, low cost, easily assessable and disposable natural sorbents derived from plants can be an alternative.

We tested different natural sorbents for extraction of PEs in milk samples. Inspired from its better extraction efficiency and highly fibrous, heterogeneous and naturally functionalized surface, seed powder of *Moringa oleifera* (*M. oleifera*) was selected as sorbent. *M. oleifera* belongs Moringaceae family and it grows in many countries. It is an environment friendly, non-toxic and biodegradable sorbent [26]. A recent review article describes potential of *M. oleifera* for treatment of water and wastewater [27]. In addition, it has been widely used for removal of metals [28] [29] and dyes [30] in aqueous solutions. However, in few studies, it has been employed for analytical extractions [31]. To best of our knowledge, this is the first report where seed powder of *M. oleifera* is used as sorbent for extraction of PEs.

2. Experimental

2.1. Chemicals and materials

The mixture of PE standards was purchased from Restek (Bellefonte, PA, USA). Following 13 compounds were considered for analysis from the mixture: dimethylphthalate (DMP), diethylphthalate (DEP), diisobutyl phthalate (DIBP), di-n-butylphthalate (DNBP), bis(2-methoxyethyl)phthalate (BMEP), bis(4-methyl-2-pentyl)phthalate (BMPP), bis(2-ethoxyethyl)phthalate (BEEP), dipentylphthalate (DPP), di-n-hexyl phthalate (DNHP), benzyl butyl phthalate (BBP), bis(2-n-butoxyethyl) phthalate (BBEP), dicyclohexyl phthalate (DCHP), bis(2-ethylhexyl)phthalate (BEHP). HPLC-grade toluene, acetone, acetonitrile, carbon tetrachloride and n-hexane were purchased from Tedia Company (Fairfield, OH, USA). Ultrapure water was produced on a Siemens Ultra Clear water purification system. Q3/2 Accurel 2E HF (R/P) polypropylene (PP) membrane sheet (157 μm thickness, 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany) and used for fabricating envelopes for the μ-SPE device. Cellulose acetate (0.2 μm pore size, 47 mm diameter), polytetrafluoroethylene (PTFE) (0.2 μm pore size, 47 mm diameter) and polycarbonate (0.2 μm pore size, 47 mm diameter) were purchased from Advantec (Toyo Roshi Kaisha, Japan). C_{18} was obtained from Alltech (Deerfield, USA). Dried *M. oleifera* seeds were purchased from local market (Alkhobar, Saudi Arabia) and ground to powder before using as sorbent. Banana peels were properly dried and powdered to use as sorbent and rice husk was obtained by a rice mill in India.

2.2. GC–MS analysis

Analysis was carried out using Shimadzu QP2010 GC–MS system equipped with a Shimadzu AOC-20i autosampler and Rxi -5 Sil MS (Restek) fused silica capillary column (30 mm × 0.25 mm

internal diameter, 0.25 μm film thickness). High purity helium (99.9999%) was used as carrier gas at a flow rate of 1.01 mL/min. Samples were injected in splitless mode. The injector temperature was set at 250 °C and the interface temperature maintained at 220 °C. The GC oven was initiated from 40 °C, and was then increased to 200 °C at 8 °C/min, and held for 1 min, after that it was increased to 220 °C at 5 °C/min, and held for 1 min, and finally it was increased to 250 °C at 3 °C/min. First, a standard solution containing high concentrations of all analytes was run in scan mode for qualitative analysis and analyte peaks were detected and confirmed using MS library (Figure S1). The analytes were analyzed in selective ion monitoring mode for quantitative determination. The monitored ions were selected based on good selectivity and high sensitivity, selected m/z values are listed in Table 1.

2.3. Preparation of natural sorbent and its characterization

Natural sorbent was obtained by grinding the seeds of *M. oleifera* to fine powder. This powder was then passed through 75 μm sieve and washed with deionized water for several times to remove any exterior contamination. After washing, sorbent was dried in oven at 60 °C for 8 h. Field emission-scanning electron microscopy (FE-SEM), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were used to characterize the sorbent.

2.4. Sample preparation

A stock solution (1000 μg L^{-1}) was prepared by diluting the standards mixture of PEs with n-hexane, and was stored in the refrigerator at 4 °C. Working standard solutions (50 μg L^{-1}) were freshly prepared by diluting the stock solution appropriately. Milk samples used for studying the extraction performance were prepared by spiking PEs free milk at known concentrations. Different brands of bottled and tetra-pack milk were purchased from the local market and stored at 4 °C till the analysis.

2.5. μ-SPE

The μ-SPE device was constructed by packing natural sorbent in a PP envelope. Fabrication of PP envelope to serve as μ-SPE device was based on very simple procedure. Briefly, the envelope was fabricated by folding the equal lengths of membrane over each other and open ends were heat sealed except the one end which was left for packing of the sorbent. 30 mg of natural sorbent was packed via the remaining open end, which was then heat-sealed to produce the extraction device (2.0 cm × 0.7 cm). The dimensions of the μ-SPE device ensured that it could fit into a 600 μL glass vial during the desorption process. Fabrication of a single μ-SPE device took 3–4 min and about 15–20 devices could be fabricated within 1 h. Each device was first conditioned and cleaned by dipping in acetone and ultrasonicating for 10 min. After conditioning μ-SPE devices were air dried.

For extraction, the μ-SPE device and magnetic stirrer were placed in a 5 mL sample solution and vortexed for 10 min. After extraction, using a pair of tweezers, the μ-SPE device was removed, dried with lint free tissue, and then fitted into a glass vial for desorption. Analytes were desorbed by adding 100 μL of acetonitrile into glass vial. Desorption was assisted by ultra-sonication for 5 min. After that, μ-SPE device was taken out of the glass vial and 1 μL of extracted liquid was injected into GC–MS. The schematic of extraction procedure is shown in Fig. 1.

2.6. Optimization of extraction conditions, calibration plots and analysis of real samples

Factors that affect extraction efficiency of μ-SPE were optimized. These factors included membrane selection, selection of sorbent and its amount, desorption solvent and its volume, extraction and desorption times, effect of salt addition. Carryover effects and reusability of the μ-SPE device were also tested. All the optimization experiments were performed using 5 mL sample solution containing 50 μg L^{-1} of PEs.

6-point calibration plots were drawn under optimum extraction conditions by using PEs free milk spiked at 1, 5, 10, 25, 50 and 100 μg L^{-1}. Finally, the method was adopted for extraction and quantitation of PEs in three different brands of milk samples.

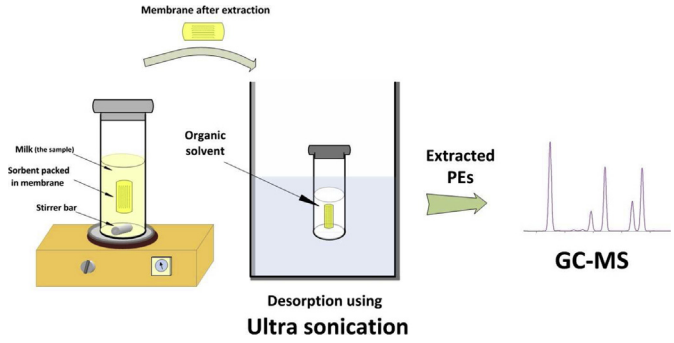

Fig. 1. Schematic of extraction method using μ-SPE. For clarity, schematic not drawn to scale.

Table 1
List of target compounds, their CAS numbers and chromatographic parameters.

Analyte	CAS number	Retention time	Target ions m/z for SIM mode	Elution order
DMP	131-11-3	15.875	163, 77	1
DEP	84-66-2	18.050	149, 177	2
DIBP	84-69-5	22.145	149,57,223	3
DNBP	84-74-2	23.735	149,104	4
BMEP	117-82-8	24.340	59,149,207	5
BMPP	146-50-9	25.575	149,85, 167	6
BEEP	605-54-9	26.385	45, 72, 149, 104	7
DPP	131-18-0	27.185	149,237	8
DNHP	84-75-3	31.260	149,251	9
BBP	85-68-7	31.440	149, 91, 206	10
BBEP	117-83-9	34.120	57, 149, 193	11
DCHP	84-61-7	35.140	149, 167	12
BEHP	117-81-7	35.645	149, 167, 279	13

3. Results and discussion

3.1. Characterization of natural sorbent

Surface morphology is critical in determination of the sorption properties of the sorbents. FE-SEM images were used to inspect surface morphology. Highly porous framework of interconnected fibers was observed by SEM images which reveals the potential of material as sorbent (Fig. 2). Presence of various functional moieties was confirmed by FTIR spectra of seed powder of *M. oleifera* (Fig. 3). A peak of OeH stretching found at 3429 cm^{-1} corresponds to proteins, fatty acids, carbohydrates and lignin units. Because of high protein content, same region is also attributed to NeH stretching of amides. Peaks at 2924 and 2853 cm^{-1} correspond respectively to asymmetric and symmetric stretching of CeH in CH_2 group. Peaks at 1710 and 1653 cm^{-1} are related to C]O stretching, which is present in proteins and fatty acid structures. Hence, the functional groups enriched bio-sorbent is supposed to interact with target compounds through above said functional moieties. FTIR spectra of banana peel and rice husk are shown in Figure S2 and S3.

The XRD patterns of the sorbent showed a single and poorly resolved broad peak indicating the amorphous nature of the material (Figure not shown). TGA and DSC were performed using SDT Q600 analyzer (TA instruments, USA) to characterize the stages of decomposition and the thermal stability of the sorbent. Both techniques present the mass loss curve of the bio-sorbent material. TGA curve (Fig. 4a) indicates that the sorbent consist of a mixture of several components confirming the heterogeneous nature. The three main stages in the mass loss curve are: (I) from 30 to 128 °C, 8% of mass loss related to water desorption, (II) from 128 to 268 °C, 32% of mass loss occurs due to decomposition of organic matter and protein components; (III) from 268 to 541 °C the greater decomposition part of the seed constituents was observed, most probably including fatty acids such as oleic acid. At 950 °C, decomposition occurred due to the presence of some inorganic oxides and ash. These facts were also supported by DSC curve (Fig. 4b).

3.2. Optimization: Factors affecting extraction efficiency

The most frequent problem that has been reported to encounter while analysis of PEs is contamination of PEs from the glassware, reagents and plastic materials in the lab. There is a probability of contamination during any step of analysis. Hence, in order to avoid the contamination of PEs, glassware was washed with ultrapure water, acetone and methanol and then dried at 120 °C overnight. This glassware was then stored in a thermally treated aluminum foil. The ultrapure water was extracted by proposed method and then desorbed by toluene. The resulting chromatogram showed some PE peaks. In order to find the source of these peaks, blank toluene was also injected into GC–MS. The concentrations of PEs in toluene was same as found in ultrapure water. Hence, the source was toluene.

Other solvents employed during this extraction were also tested for contamination and PE traces were also found in carbon tetrachloride. No traces of PEs were found in acetonitrile and n-hexane. The solvents toluene and carbon tetrachloride were also investigated during solvent optimization step, however, the initial contamination was taken into account while quantitation of PEs. The best extraction performance was obtained with acetonitrile. As initial screening showed that it was free of PE traces, hence, no additional calculations were needed for all upcoming optimization procedures.

Optimization was carried out by analysis of triplicate uncontaminated milk samples spiked with 50 μg L^{-1} of PEs.

3.2.1. Membrane selection

We tested different types of commercially available porous membranes PP, polycarbonate, cellulose acetate and PTFE in order to contain sorbent materials for μ-SPE. Porous polypropylene membrane was chosen as the most suitable membrane for making μ-SPE devices. The wide chemical compatibility of PP membrane makes it an appropriate membrane for μ-SPE device. Other membranes such as polycarbonate, cellulose acetate and PTFE were deemed unsuitable. The polycarbonate membrane was flimsy whereby vigorous stirring during extraction would tear the device even before the completion of extraction. Cellulose acetate membrane was brittle and could snap upon folding during the preparation of μ-SPE device. PTFE membrane was not heat sealable.

3.2.2. Sorbent selection and amount

The selection of the sorbent is of vital importance for μ-SPE because it determines selectivity towards analytes. Extraction efficiency of seed powder of *M. oleifera* was compared with C_{18}, banana peel and rice husk. C_{18} is commonly used conventional sorbent in μ-SPE for extraction of organic analytes [32] while banana peel and rice husk are among other commonly used bio-

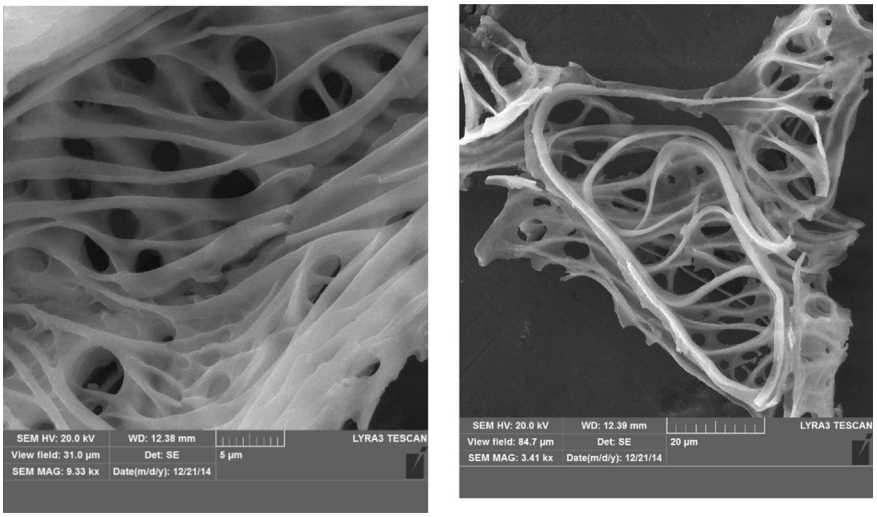

Fig. 2. SEM images of natural sorbent (Seed powder of *M. oleifera*) at different magnifications.

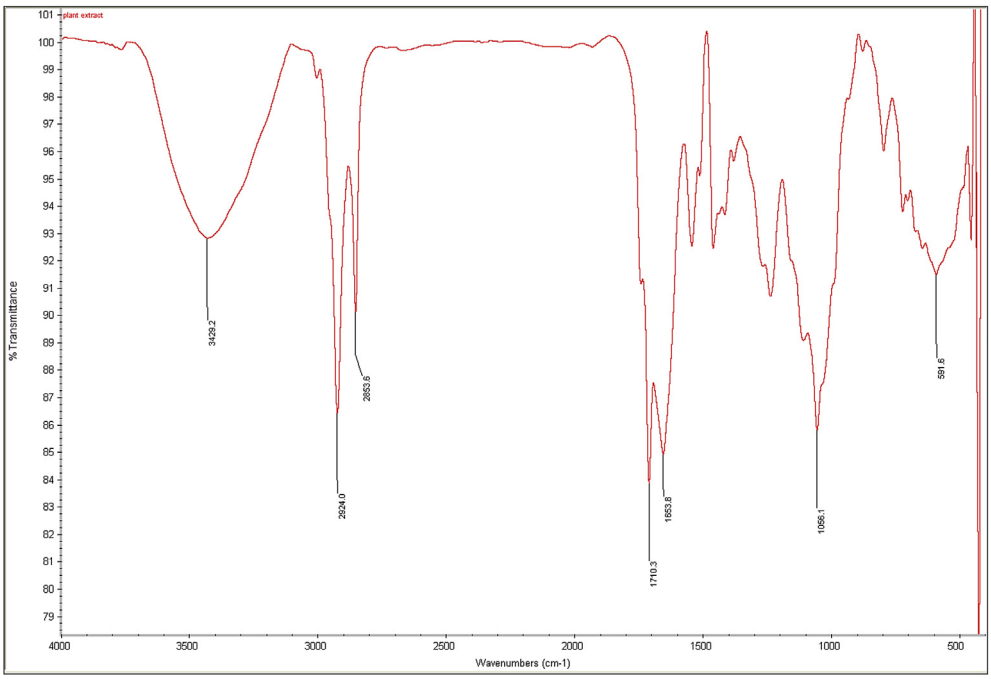

Fig. 3. FTIR spectrum of seed powder of *M. oleifera*.

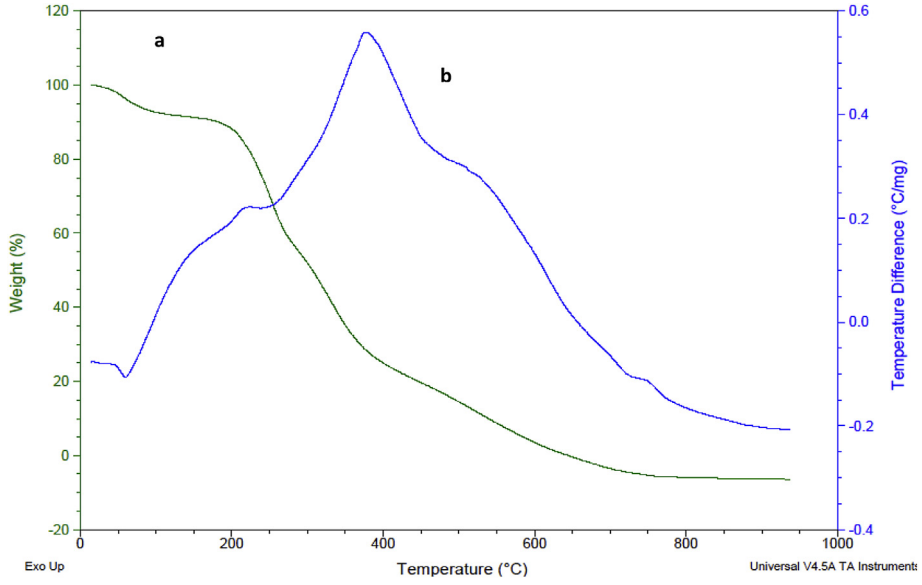

Fig. 4. TGA (a) and DSC (b) of seed powder of *M. oleifera*.

sorbents. The constant mass of each sorbent (50 mg) was used for selection of optimum sorbent. Seed powder of *M. oleifera* was found to be most effective sorbent and gave highest peak areas compared to other sorbents (Fig. 5). *M. oleifera* seeds are reported to contain significant fiber, protein and ash content [33]. All these properties are advantageous for adsorption of target compounds. Hence, better extraction performance of *M. oleifera* may be attributed to.

(i) Its fibrous structure which comprise a highly porous network of interconnected fibers. In general, fibrous structures have good adsorption capabilities.
(ii) Presence of proteins and other naturally functionalized moieties that may interact with target compounds. Larger the amount of proteins in a bio-sorbent corresponds to abundance of functional groups. The overall protein content of *M. oleifera* seeds is reported up to 38% [34] and it is much higher than the protein content of banana peel (0.90%) [35] and rice husk (2.5%) [36].
(iii) Electrostatic interactions between carbon content and target compounds.

Hence, *M. oleifera* was selected as sorbent for further experiments.

Different amounts (20, 30, 60, 90, 120 mg) of natural sorbent were packed into polypropylene membrane and 30 mg was found as an optimum sorbent amount. Peak areas increased up to 30 mg

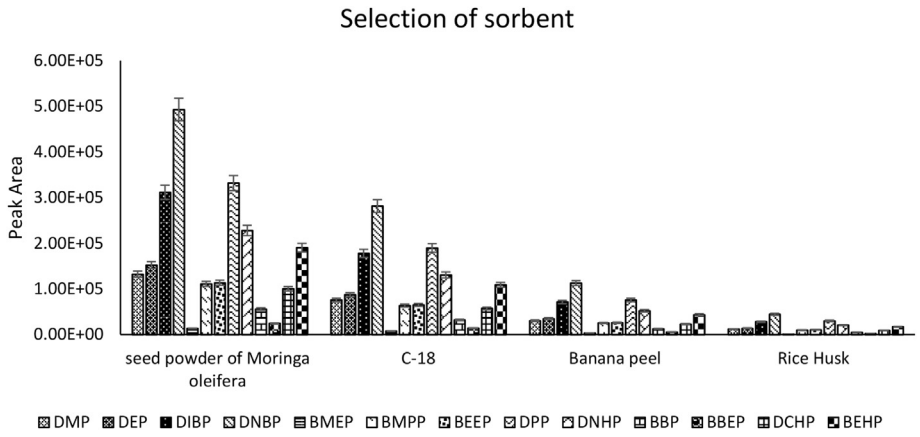

Fig. 5. Influence of sorbent type on extraction of PEs. Conditions: PEs concentration: 50 μg L^{-1}; extraction time: 10 min; desorption solvent: toluene; desorption volume: 250 μL; desorption time: 5 min; amount of salt: 0%.

and then became constant. This amount can easily fit into a desorption vial and desorption can be done using smaller volume of the organic solvent.

3.2.3. Desorption solvent and its volume

After extraction, the μ-SPE device was transferred into a glass vial. After that, desorption solvent was added to the glass insert and desorption was carried out with aid of ultrasonication. Desorption solvents with varying polarity index were investigated to get the maximum desorption of the analyte from sorbent to desorbing solvent. The solvents toluene, acetonitrile, n-hexane and carbon tetrachloride were tested. Fig. 6 shows that highest peak areas were obtained by using acetonitrile and it was selected as an optimum desorption solvent for further experiments.

A series of experiments was performed to find out the optimum volume of the desorption solvent (acetonitrile). Different volumes of acetonitrile ranging from 100 to 400 μL were investigated. The highest peak areas were obtained with 100 μL of desorption solvent. This can be attributed to the high enrichment of analytes in lower volumes. The volumes below 100 μL were not considered in order to have sufficient volume for immersion of μ-SPE device and complete desorption thereafter. Hence, 100 μL acetonitrile was adopted as an optimum value of desorption solvent (Figure S4).

3.2.4. Extraction and desorption times

The extraction efficiency of μ-SPE device depends on the rate of mass transfer of analytes from the donor phase (sample solution) to the sorbent phase. Sample agitation improves extraction efficiency by enhancing contact between the analytes and sorbent phase. Hence, extraction (agitation) time is an important parameter to consider. The extraction time was evaluated in range of 5–20 min Fig. 7 shows that peak areas of analytes increased with extraction up to 10 min with no significant increase thereafter. After 10 min, peak areas reached to a steady state. As in μ-SPE, the analytes are dynamically distributed between the sorbent and solution phase. Establishing a steady state after 10 min is indicative to mass transfer equilibrium between sorbent and solution phase. Hence, extraction time of 10 min was selected as an optimum time for next experiments.

The effect of desorption time was investigated over a range of 5–20 min. Maximum extraction efficiency was observed at desorption time of 5 min. After 5 min, extraction efficiency was gradually decreased which can be attributed to increased temperature as a result of longer periods of ultra-sonication. PEs may evaporate at increased temperatures (Figure S5).

3.2.5. Effect of salt addition

In most of the microextraction studies, experiments are performed to evaluate the effect of salt addition on extraction

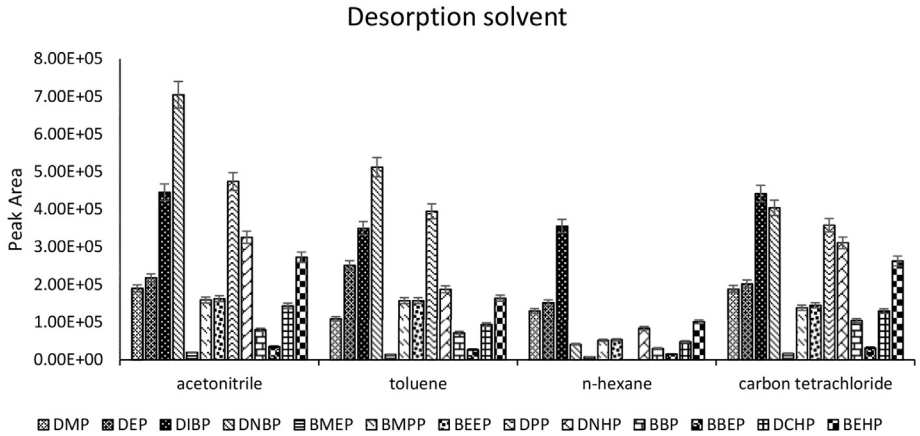

Fig. 6. Influence of desorption solvent on extraction of PEs. Conditions: PEs concentration: 50 μg L^{-1}; extraction time: 10 min; desorption volume: 200 μL; desorption time: 5 min; amount of salt: 0%.

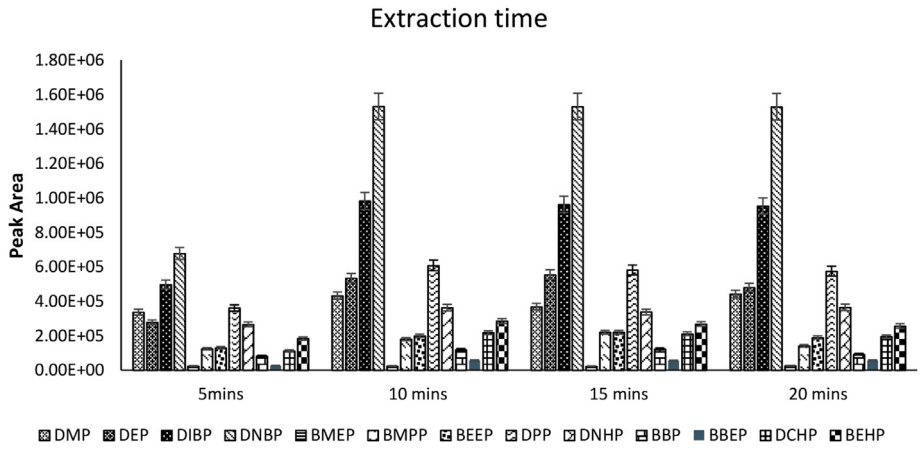

Fig. 7. Influence of extraction time on extraction of PEs. Conditions: PEs concentration: 50 μg L^{-1}; desorption solvent: acetonitrile; desorption volume: 100 μL; desorption time: 5 min; amount of salt: 0%.

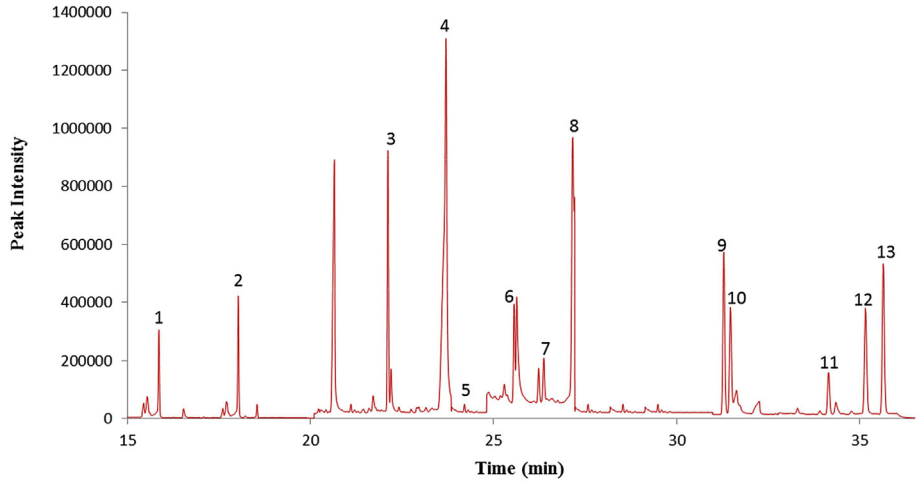

Fig. 8. GC–MS trace of a spiked real milk sample extracted under most favorable μ-SPE conditions. (1) DMP, (2) DEP, (3) DIBP, (4) DNBP, (5) BMEP, (6) BMPP, (7) BEEP, (8) DPP, (9) DNHP, (10) BBP, (11) BBEP, (12) DCHP, (13) BEHP.

efficiency. Upon salt addition, solubility of the target analytes decreases in the aqueous phase and it leads to their migration to extractant phase. Salting-out effect was studied by adding various amounts of NaCl to milk samples (ranging from 0 to 20%, w/v). The highest peak areas were obtained at 0% (without salt addition) (Figure S6).

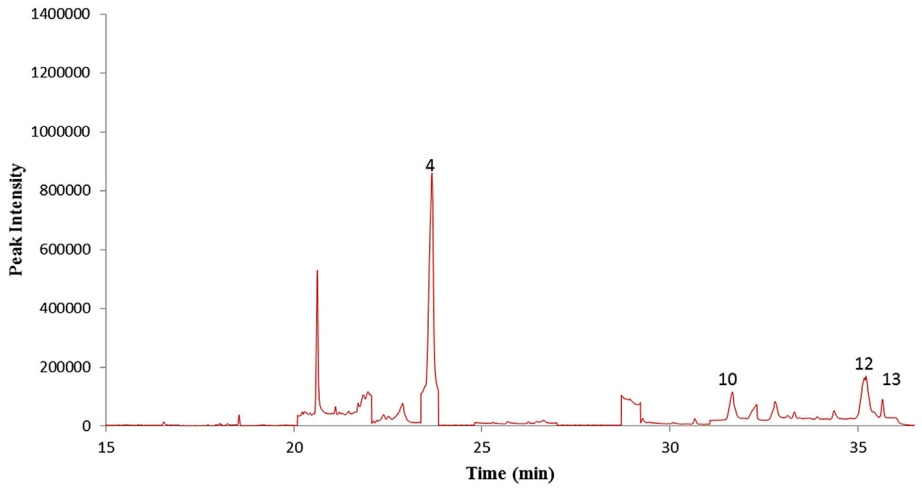

Fig. 9. GC–MS trace of a real milk sample extracted under most favorable μ-SPE conditions. Detected compounds are: (4) DNBP, (10) BBP, (12) DCHP, (13) BEHP.

Upon addition of 5% salt, we observed that peak areas of some analytes were significantly decreased while for other compounds they remained constant. Further addition of salt up to 20% did not affect peak areas. This decrease in extraction efficiency upon salt addition can be due to enhanced viscosity of the sample solution which hinders movement of target analytes from donor to acceptor phase [37]. Moreover, salt addition will precipitate proteins which may hinder adsorption of target compounds into μ-SPE device.

3.2.6. Carry over effects and reusability of μ-SPE device

After the first desorption round, the μ-SPE device was re-desorbed in acetonitrile to investigate carryover effects. No analyte peaks were detected which meant that device can be re-used. Hence, we investigated the repeated use of μ-SPE device and results confirmed that it could be used for 20 to 25 times. RSD values for the same device was below 7% and with different μ-SPE devices, the RSDs ranged between 3.8 and 9.2%. The reusability of μ-SPE device is, however, highly dependent on the durability of membrane material itself.

3.3. Method validation

To evaluate analytical performance of the proposed natural sorbent based μ-SPE-GC-MS method, parameters like linearity, repeatability, limits of detection and enrichment factors were investigated under optimum experimental conditions. The results are summarized in Table 2.

Calibration curves were plotted under most favorable extraction conditions using milk samples that were originally free from PEs and spiked with different concentrations of PEs (1, 5, 10, 25, 50, 100 μg L^{-1}) (Figure S7). Each point of the calibration curve corresponded to the average value obtained from seven measurements. Good linearity was observed over a concentration range of 1–100 μg L^{-1} with coefficient of determination (R^2) ranging from 0.9768 to 0.9977. LODs calculated based on signal-to-noise (S/N) ratio of 3, were in the range of 0.01–1.2 μg L^{-1}. Precision of any developed method is determined by reproducibility data. Intra-day and inter-day reproducibility was accessed by extracting the spiked milk samples (n = 7 for each) under the same extraction conditions and the values of relative standard deviations (RSDs) were in between 3.6% and 10.2%. This reflects an acceptable precision. Similarly, repeatability of extraction was studied with different μ-SPE devices and satisfactory RSD values ranging between 2.5% and 7.2% were obtained.

Table 3
Study of matrix effect on extraction of PEs from different brands of milk samples.

Mean relative recovery ± standard deviation (n = 3)						
Analyte	Spiked at 25 μg L^{-1}			Spiked at 50 μg L^{-1}		
	Brand 1	Brand 2	Brand 3	Brand 1	Brand 2	Brand 3
DMP	102 ± 3	99 ± 4	101 ± 4	101 ± 5	99 ± 2	100 ± 1
DEP	98 ± 2	100 ± 3	99 ± 6	102 ± 2	98 ± 5	99 ± 4
DIBP	98 ± 3	99 ± 6	94 ± 4	100 ± 2	99 ± 3	96 ± 3
DNBP	97 ± 2	98 ± 5	100 ± 4	99 ± 2	96 ± 6	101 ± 1
BMEP	99 ± 5	102 ± 4	100 ± 3	97 ± 1	103 ± 4	101 ± 2
BMPP	96 ± 5	96 ± 7	93 ± 5	98 ± 4	97 ± 6	98 ± 5
BEEP	89 ± 8	92 ± 7	95 ± 4	90 ± 9	92 ± 6	97 ± 5
DPP	91 ± 7	88 ± 8	94 ± 3	93 ± 9	91 ± 8	96 ± 4
DNHP	86 ± 11	89 ± 9	85 ± 4	88 ± 5	92 ± 5	88 ± 3
BBP	89 ± 9	98 ± 3	82 ± 8	91 ± 8	101 ± 2	83 ± 4
BBEP	78 ± 5	79 ± 4	82 ± 5	79 ± 6	81 ± 4	85 ± 7
DCHP	81 ± 6	79 ± 5	85 ± 2	82 ± 7	78 ± 4	83 ± 5
BEHP	79 ± 5	77 ± 9	83 ± 6	80 ± 4	78 ± 5	81 ± 2

Table 4
Concentrations of PEs in real milk samples.

Analyte	Concentrations of PEs (μg L^{-1}) in Real milk samples		
	Brand 1	Brand 2	Brand 3
DMP	ND	25.1	ND
DEP	ND	ND	ND
DIBP	ND	ND	ND
DNBP	32.3	6.3	19.2
BMEP	ND	ND	12.5
BMPP	ND	ND	ND
BEEP	ND	ND	ND
DPP	ND	15.2	ND
DNHP	ND	ND	ND
BBP	15.8	ND	ND
BBEP	ND	ND	ND
DCHP	18.8	ND	ND
BEHP	8.9	10.2	ND

ND = not detected.

3.4. Real samples

The proposed method was applied for determination of PEs in the milk samples. For this purpose, three different brands of milk samples (2 bottled and 1 tetra pack) each with fat content of 3% were obtained. In order to access the matrix effect, the relative recoveries of the method were calculated by extracting the spiked milk samples and comparing with extraction of the same

Table 2
Analytical features of proposed method.

Compound	DLR[a] (μg L^{-1})	Coefficient of determination (r^2)	LOD[b] (μg L^{-1})	LOQ[c] (μg L^{-1})	RSD[d](%) (n = 7)	
					Intra-day	Inter-day
DMP	1–100	0.9977	0.07	0.22	5.4	6.7
DEP	1–100	0.9966	0.06	0.18	4.1	4.5
DIBP	1–100	0.9973	0.04	0.12	8.7	9.1
DNBP	1–100	0.9928	0.01	0.4	6.0	5.7
BMEP	5–100	0.9908	1.2	3.7	7.6	8.3
BMPP	1–100	0.9943	0.07	0.21	3.6	5.8
BEEP	1–100	0.99	0.09	0.27	5.9	7.2
DPP	1–100	0.9914	0.03	0.10	7.0	6.7
DNHP	1–100	0.9768	0.06	0.18	4.6	4.3
BBP	1–100	0.9949	0.06	0.19	4.7	5.9
BBEP	1–100	0.9898	0.11	0.34	8.4	7.8
DCHP	1–100	0.9926	0.08	0.24	6.2	4.7
BEHP	1–100	0.9843	0.07	0.21	9.4	10.2

[a] Dynamic linear range.
[b] Limit of detection: calculated as three time of the baseline noise.
[c] Limit of quantitation: 3 × LOD.
[d] Reproducibility.

Table 5
Comparison of the proposed method with others reported in the literature.

Method	Number of PEs studied	LODs (μg L^{-1})	Linear range (μg L^{-1})	Extraction time (min)	Sample volume (mL)	Reference
SPE-GC-MS	5	0.06–0.12	–	–	–	[6]
DLLME-GC-FID	5	0.5–3	50–800	15	5	[5]
LE-SPE-LC-MS/MS	6	0.01–0.5	–	100	3	[39]
LLE-SPE-LC-MS/MS	5	5–9		LLE and SPE needs extraction, drying, reconstitution and clean up. Huge time consumption	Large volumes of organic solvents utilized.	[40]
μ-SPE-GC-MS	13	0.01–0.20	1–100	15	5	Present work

concentrations spiked in water samples. The mean recoveries for all analytes in three different brands of milk samples are listed in Table 3. Some differences in the recoveries were observed such as long alkyl chain containing PEs e.g. BBEP and BEHP showed relatively low recoveries. It can be explained by two ways.

(i) As the sorbent is naturally functionalized with polar moieties, it will interact strongly with relatively polar PEs. As the length of alkyl chain increases in PEs, their polarity will decreases, which in turn leads to their relatively poor extraction by the sorbent.
(ii) Secondly, it is probably an indication that matrix effect is less effective in case of relatively polar PEs but as the length of alkyl chain in PE molecules increases, the interactions between PEs and proteins in the milk become more prominent. Similar findings have also been reported in another study [5].

Only seven compounds DMP, DNBP, BMEP, DPP, BBP, DCHP and BEHP were detected in some of the real milk samples (Table 4). Maximum contamination levels (MCLs) of PEs in milk have not been regulated yet, but the MCLs for DEHP in water is 6 μg L^{-1} [38]. The concentrations found in milk samples were higher than this level. Figs. 8 and 9 show GC-MS traces of spiked and unspiked real milk samples respectively.

The performance of the proposed method was compared with those reported in the literature (Table 5). The natural sorbent based μ-SPE-GC-MS method has several advantages on the methods reported in literature. It requires small volume of sample (5 mL). In addition, it utilizes very small amount of the natural sorbent (30 mg) compared to conventional SPE. The proteins and fats from the milk samples cannot deposit over the sorbent as it is protected inside the membrane by heat sealing. No additional steps are needed for removal of proteins from the milk prior to extraction, which is usually time extensive. Moreover, very small volume (100 μL) of the organic solvent is required for desorption of the target analytes from the sorbent, which is a unique advantage in terms of green methods. A single μ-SPE device can be reused for 20–25 times before any signs of wear and tear appears on it. Similarly, very good values of LODs and linear range make this method comparable or superior than the reported methods. All this discussion shows that proposed method is rapid, and efficient for determination of PEs in the milk samples.

4. Conclusion

In the present study, a natural sorbent based μ-SPE was utilized for the extraction of PEs in the milk samples. Efficient extraction of PEs was originated from the unique properties of the natural sorbent such as porous and fibrous structure and natural enrichment with functional moieties. *M. oleifera* as sorbent is easy to use, reuse and dispose of. It is green, low cost and readily available sorbent. The proposed natural sorbent based μ-SPE method gave reasonable reproducibility and good linearity with LODs lower or comparable to the methods reported in the literature.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.04.016.

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