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Optimization of microwave-assisted extraction followed by solid phase micro extraction and gas chromatography-mass spectrometry detection for the assay of some semi volatile organic pollutants in sebum

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Abstract

Methodology using MAE/SPME/GC–MS is being pursued for the analysis of organic pollutants in sebum. The microwave-assisted extraction (MAE) of standards of semi volatile organic pollutants from sebum was optimized. All compounds were extracted from sebum with recoveries analyzed by GC/MS ranging from 94% to 100% under the optimum MAE conditions: 10 mL acetone–hexane (2:1), 60 °C, and 10 min microwave heating. To improve the detection limits a SPME procedure was optimized. Linearity ranged from 0.70 ppb to 25 ppb. R.S.D. were in the range of 1–23% for the SPME step. Preliminary real samples were analyzed and a range of compounds was detected. The optimized MAE/SPME/GC–MS methodology promises to be useful for different applications.

Keywords: Sebum; Organic pollutants; Microwave-assisted extraction; Solid phase microextraction; GC–MS

1. Introduction

The pollution levels of our world are increasing continuously with time. Puerto Rico is not an exception; we have areas with a high incidence of pollution due to incinerators, thermoelectric plants, and a strong history of heavy manufacturing among other factors. We are exposed to an overwhelming number of chemical contaminants every day in our air, water, food, and general environment. The bioaccumulation of pollutants in the human body can contribute to the development of several diseases. Concern about pollutants (e.g. poly aromatic hydrocarbons (PAHs), dioxin) initially focused on their ability to cause cancer, but more recently concern has turned to their interference with hormones systems and their potential effects on reproduction, as well as their ability to depress immune function [1].

Semi volatile organic pollutants, especially those that are persistent (POPs), have been shown to exhibit potentially harmful effects to the environment and human beings. Many of the chemicals that we use daily can be harmful to our health. As an example pesticides used in agricultural as well as industrial or home pest control should be considered as hazardous chemicals with great concern for the general population. To varying degrees semivolatile organic pollutants, resist photolytic, biological and chemical degradation and tend to accumulate in living tissues. In the case of POPs, their persistence in various media facilitates their transport over long distances to remote regions where they have never been used. POPs are primarily products and by-products of human enterprise. Sources include pesticide applications, chemical manufacturing, combustion and incineration (i.e.

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PAHs, PCDDs, PCDFs) [2]. Most of the POPs are toxic, lipophilic, and semivolatile. The combined characteristics of being lipophilic and persistent make biomagnification and bioaccumulation a major concern. It is well known that some organic pollutants accumulate in the fatty tissues of animals and people (i.e. dioxin and congeners) and they are excreted slowly from feces, sebum and breast milk [3]. The metabolism of certain families of organic compounds have been well studied in experimental animals, however, human data is limited [4–6]. Their determination and study of human exposure could allow the identification of some causes and symptoms of health conditions. This could lead to a better understanding of substance interactions with the environment and the process of incorporation into the human body. The measurement of organic pollutants in different biological matrices provides insight into mechanisms of organic pollutants in the body. Blood [7], hair [8], urine [9], serum [10], breast milk [11] and adipose tissue [12] are among the most frequently used matrices to monitor exogenous compounds in humans.

Sebum is an oily substance comprised primarily of lipids including glycerides, free fatty acids, wax esters, squalene, cholesterol esters and cholesterol [13]. It is produced in cells of sebaceous glands. The excretion of organic pollutants in sebum has received only limited study [3]. Based on studies already done, we can see that sebum is a promising matrix to diagnose exposure to a wide range of exogenous substances like codeine and cocaine [14], fluconazole [15] and dioxins [16]. Considering the liphophilicity of some semi volatile organic pollutants, it seems likely that their secretion in sebum could represent an important route for their elimination from the body.

The aim of this study is the development of noninvasive methodology for the diagnosis of possible exposure to environmental pollutants through sebum. The parameters of sample collection, extraction, and analysis are being studied in our development of methodology. Sebum is easily collected by several noninvasive techniques including the placement of cigarette rolling paper against the skin, the application of a lipid-absorbent tape (Sebutape®, CuDerm Corp. Dallas TX) to the skin surface, the application of an absorbent, opalescent film to the skin and through wiping of the skin with cotton or cotton swabs [17]. Sebutape was chosen as the collection device for the present study. Collection of sebum samples with Sebutapes® is a simple and widely applied technique in dermatological studies. Sebutapes are white, open-celled, micro porous, hydrophobic films of polypropylene coated with an adhesive layer, with pores specifically designed to collect sebum [17]. Sebum is collected and accumulated passively in the sebutape pores and appears as translucent spots.

The analyses of some exogenous substances in sebum until now have been performed using sonication extraction, liquid–liquid, liquid–solid extractions and mechanical shaking. Since these conventional techniques are laborious, time consuming and need large volumes of toxic organic solvents, much attention in our study is being paid to the development of more efficient environmentally friendly techniques for the rapid analytical-scale extraction of exogenous compounds from sebum, such as microwave-assisted extraction (MAE). This technique, MAE, consists of heating the media, mostly liquid organic solvents in contact with the sample with microwave energy. This extraction technique in the past few years has been successfully applied to extract organic pollutants from different matrices, such as PAHs, pesticides (i.e. organochlorinated, organophosphorous, sulfonylureas, triazines), PCBs and phenols [18]. Some of the matrices from which organic pollutants have been extracted are soils, sediments, vegetables, water samples and animal tissues. In these studies, MAE was more effective and reduced the sample preparation time and solvent volumes substantially in comparison with conventional extraction techniques like Soxhlet, liquid–liquid extraction and sonication [19]. Although MAE has many advantages, only few studies have been reported on the MAE of biological matrices.

Solid phase microextraction is a sample preparation technique introduced by Pawliszyn and collaborators in 1989. Analytes are pre-concentrated on the surface of a polymeric coated fiber and subsequently desorbed into the injector of a chromatographic system [20]. Advantages of this extraction technique, such as simplicity and capability of injecting the entire extracted sample, have been discussed in several papers [21–23]. This technique has been used into several areas of biomedical analysis (e.g. analysis of pesticides in human fluids, analysis of drugs and its metabolites) [24].

In this study, MAE followed by SPME/GCMS analysis were optimized and used to extract some representative standards of organic pollutants from sebum. A solid phase microextraction of the MAE extract was performed in order to introduce more analytes for the GC/MS to improve the detection limits.

2. Experimental

2.1. Reagents and sebum sample preparation

For practical purposes a model matrix of sebum spiked with some persistent organic pollutants was used for the optimization of the extraction techniques. A synthetic sebum was obtained from Scientific Services (New York, USA). The standards pyrene 99%, 1,4-benzodioxan 98%, and 1,2,4,5-tetrachlorobenzene were obtained from Sigma–Aldrich (St. Louis, MO). Pyrene was used to represent one of the groups of POPs, the PAHs. In order to represent the dioxins group we chose 1,4-benzodioxan that although it is not a dioxin, its structure is very similar and is less toxic. Endrin, dieldrin and aldrin were acquired from Poly Science Corp. (Niels, IL, USA). These along with 1,2,4,5-tetrachlorobenzene were used to represent the organochloride pesticides group. Dursban was used in representation of organohosphate pesticides (Poly Science Corp., Niesls, IL, USA). Also Permethrin (Poly

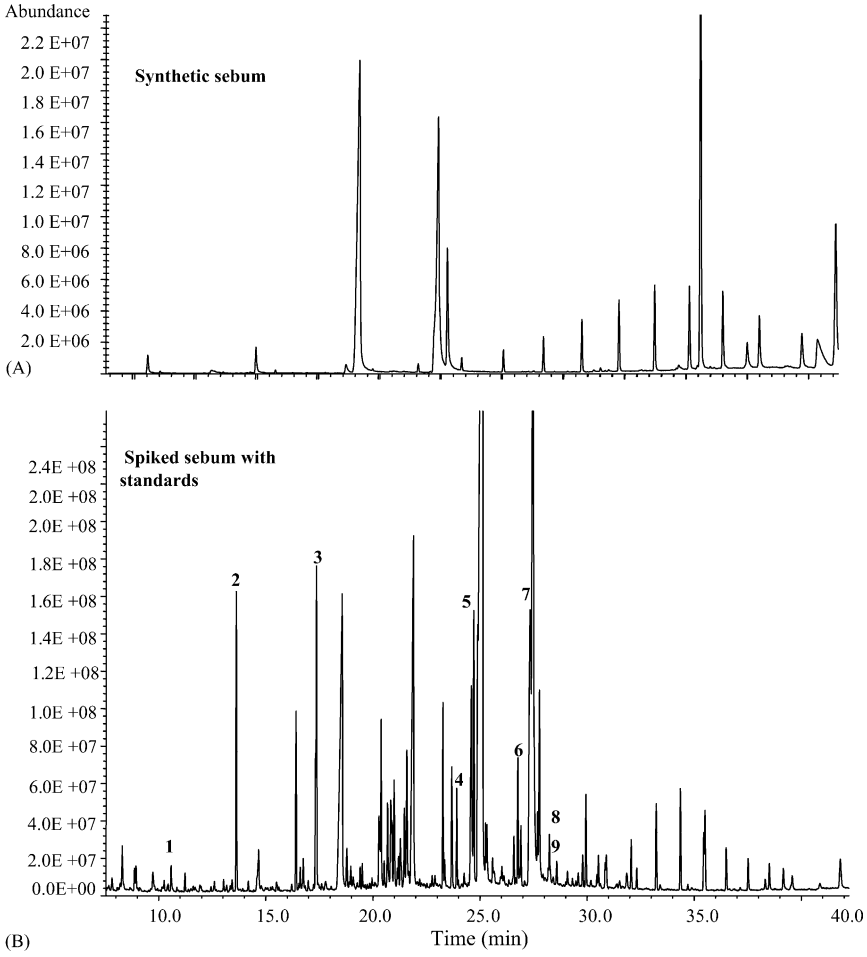

Fig. 1. (A) Chromatogram obtained by GC/MS of synthetic sebum (direct injection). (B) Chromatogram obtained by GC/MS after SPME of an aliquot of sebum model matrix spiked with standards in 5 mL of water (25 ppb): (1) 1,4-benzodioxan; (2) 1,2,4,5-tetrachlorobenzene; (3) benzofuran; (4) aldrin; (5) dursban; (6) pyrene; (7) endosulfan; (8) dieldrin; (9) endrin.

Science Corp., Niesls, IL, USA) was used as a representative standard of the pyrethroids pesticides. A stock solution of the model matrix was prepared as follows: the synthetic sebum was dissolved in dichloromethane and the standards of interest were spiked into the solution. An aliquot of 3 μL of the spiked sebum solution was taken, deposited on the sebutapes® and the solvent allowed to evaporate. The final concentration of the standards, if a 100% of recovery was obtained in the MAE, was 3 ppm. For each extraction that was performed one spiked sebutape was used. Sebutapes® were obtained from CuDerm Dallas Texas. For the SPME optimization, an aliquot of model matrix spiked with the standards was diluted in nanopure water (final concentration of the standards was 25 ppb). Fig. 1 shows representative chromatograms of synthetic sebum and spike sebum with typical concentrations of some analytes. All the solvents, acetone, hexane, methanol and acetonitrile (optima) were from Fisher Scientific (Fair Lawn, NJ, USA). Nanopure water was obtained from a Barnstead purification system (mega-PureÓ System MP-3A with NANOpure ultrapure water system.

2.2. Instrumentation

2.2.1. Microwave-assisted extractor

Microwave-assisted extraction was carried out using an Ethos Plus laboratory microwave extraction system (Milestone Inc. Monroe, Connecticut). The instrument is able to extract concurrently 10 samples under identical extraction conditions. This system was comprised of an Ethos laboratory microwave unit with a built-in magnetic stirrer and a fiber optic temperature sensor.

2.2.2. SPME

The SPME device and SPME fibers used were purchased from Supelco. Seven different fiber types were purchased: polydimethylsiloxane (PDMS, 100 μm and 7 μm), carbowax–divinylbenzene (CW/DVB, 70 μm), carboxen–polydimethylsiloxane (CAR/PDMS, 85 μm), polydimethylsiloxe–divinylbenzene (PDMS/DVB, 65 μm), polyacrylate 85 μm, and divinylbenzene/caboxen polydimethylsiloxane (DVB/CAR/PDMS 50/30 μm). Fibers were conditioned in the injector of the GC for a period of time as indicated by

the manufacturer before use. This conditioning is specific for each fiber type.

Spiked model matrix was introduced into 5 mL vials. Sample vials were completely filled with solution in order to avoid headspace. Extraction time was 45 min, extraction temperature was 40 °C and stirring rate was 1000 rpm. Samples were stirred on a ceramic top, digital stirring (±20 rpm) hotplate. The DVB/CAR/PDMS 50/30 μm fiber was selected for the pre-concentration procedure with SPME. This fiber was immersed in the sample solution and the extraction was performed applying the optimized parameters. Then analytes were desorbed in the injector port for 15 min at 260 °C. Desorption time was determined experimentally.

2.2.3. GC–MS

All the extracts were analyzed on a Hewlett-Packard 5890 Series II-GC coupled with a 5971-MS System. The system was calibrated daily and system blanks as well as known standards were run to assure optimum performance. The analysis conditions were: a J&W DB5MS column (bonded and cross-linked 5%-phenyl-methylpolysiloxane), column length: 30 m, column i.d.: 0.32 mm, film thickness: 0.25 mm). SPME inserts of 0.75 mm i.d. for Hewlett-Packard (Supleco) were used. The GC temperature program was as follow: oven was initially 50 °C for 3 min, 6 °C/min until 260 °C, and held at 260 °C for 3 min. Samples were injected in the splitless mode and a solvent delay of 4 min was allowed. The MS was in the positive electron impact mode at 70 eV and the mass detection range was 50–550 *m/z*. Carrier gas was helium at 0.9 mL/min. Standards were monitored with the full scan acquisition mode and peaks were identified with a NIST/EPA/NIH Mass Spectral library.

2.3. Microwave-assisted extraction procedure

Each extraction was performed using one sebutape spiked with 3 μl of the model matrix. The sebutapes were transferred to the Ethos extraction vessels. According to a pre-designed experimental trial, the respective solvent volumes were added into the vessels and carried out under different microwave-assisted extractions. For each set of conditions a sebutape without model matrix was extracted and used as a process blank. After extraction, the vessels were cooled down to room temperature before opening. The extract was transferred to tubes for pre-concentration. The sebutape and the extraction vessel were washed three times with 0.5 mL of the extrac-

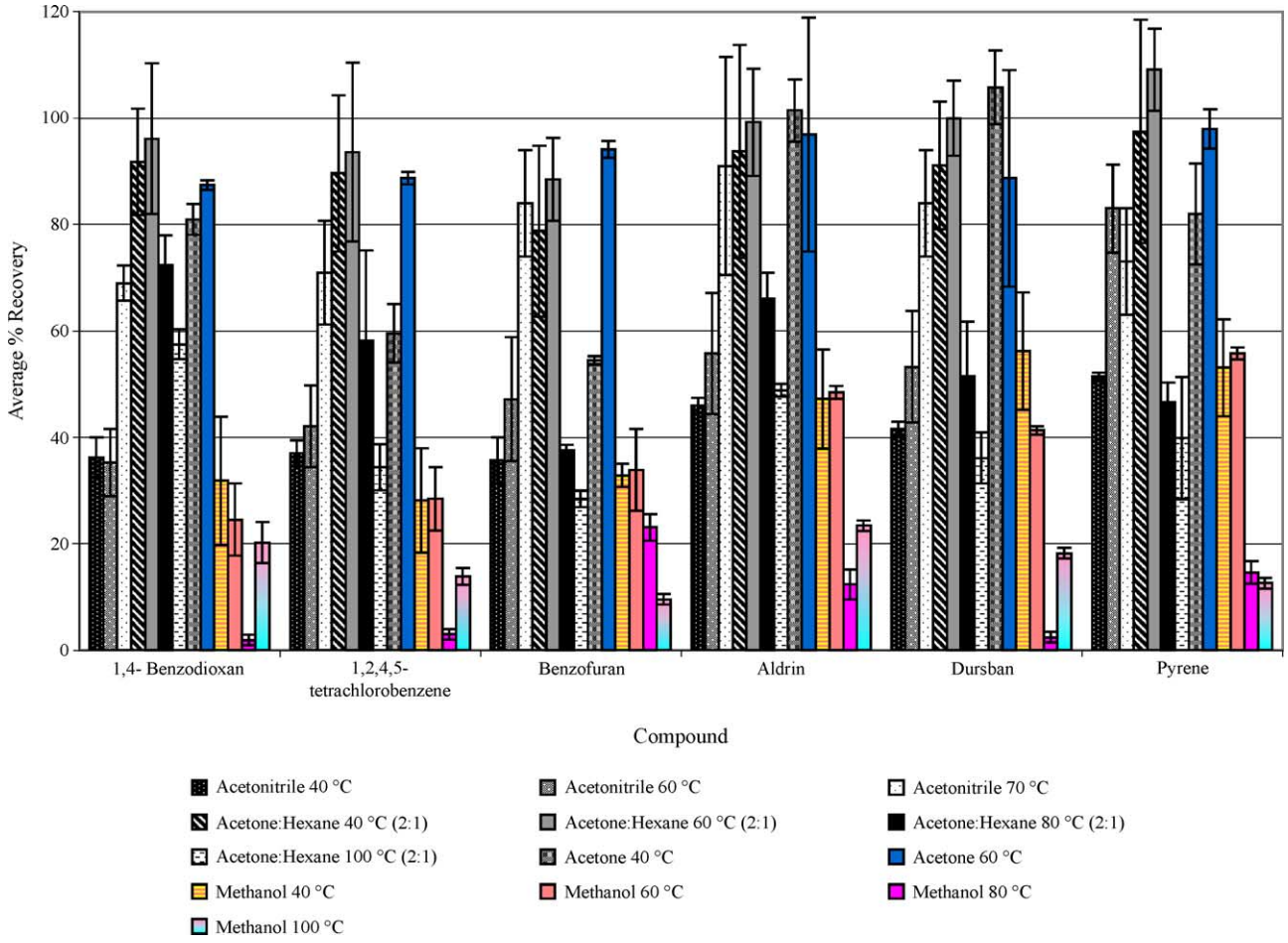

Fig. 2. Comparison of average recoveries for some representative standards using different solvents and temperatures in MAE. The extractions were performed under the following conditions: 10 min of microwave heating and 10 mL of solvent.

tion solvent and this was added to the pre-concentration tube with the extract. The extracts were pre-concentrated with an inert gas stream (N_2) to 1 mL and 1 μL of the solution was directly analyzed by GC–MS in the optimization of the MAE extraction parameters. For the SPME procedures 0.25 mL of the extract was diluted in nanopure water to 5 mL.

3. Results and discussion

3.1. Microwave-assisted extraction optimization

The main parameters under study were (1) extraction solvents; (2) extraction temperature; (3) extraction time; and (4) volume of extraction solvent. The first experiment for the optimization of MAE was designed to determine the best extraction solvent and extraction temperature. Four solvents (acetonitrile, acetone, acetone:hexane (2:1) and methanol) and different extraction temperatures in the range of 40–100 °C were tested. For each trial the first five min of the extraction the temperature was raised from 25 °C to the desired temperature and then five more min at the temperature under study. Error estimation is done by experiment repetition; each combination of solvent and temperature was performed in triplicate. Fig. 2 shows the percents of recovery that were obtained under the different solvents and temperatures of extraction.

In Fig. 2 can be seen that good recovery percentages were obtained with acetone at 60 °C, acetonitrile at 70 °C and the mixture of acetone–hexane (2:1) at 60 °C. The highest percent of recovery for most of the standards was obtained with the mixture of acetone:hexane (2:1) at 60 °C. Using these conditions the percents of recovery were between 94% and 109%. This experiment demonstrated that the identity of the extraction solvent and the extraction temperature in MAE were significant for almost all the compounds. Also the combination of the solvent and the extraction temperature is important. For most of the solvents used the best extraction temperature was near 60 °C. The mixture of acetone:hexane (2:1) at 60 °C was chosen as the best overall combination for the MAE process.

Once the mixture of acetone:hexane (2:1) at 60 °C was set as the extraction solvent and temperature, the extraction time and volume were determined. For the optimization of extraction time 10 mL of acetone–hexane (2:1) were used. Three temperature and time programs were tested: (1) 10 min of extraction [5 min for the temperature rise from 20 °C to 60 °C and 5 min of extraction at 60 °C]; (2) 15 min of extraction [5 min for the temperature rise from 20 °C to 60 °C and 10 min of extraction at 60 °C]; and (3) 20 min of extraction [5 min for the temperature rise from 20 °C to 60 °C and 15 min of extraction at 60 °C]. The results of this experiment are displayed in Fig. 3. From this graph it can be seen that for most of the compounds, an increase of the extraction time resulted in a decrease in the percent of the recovery. It has been reported that at higher extraction time in polymer matrices the percent of recovery decreases. This was explained by increased dissolution of the polymer matrix at longer extraction times, causing an increase in viscosity, which makes the matrix encapsulate the target analyte [19]. In this study, this effect could be due to the fact that at higher extraction times the solvent begins to dissolve the material of the sebutape

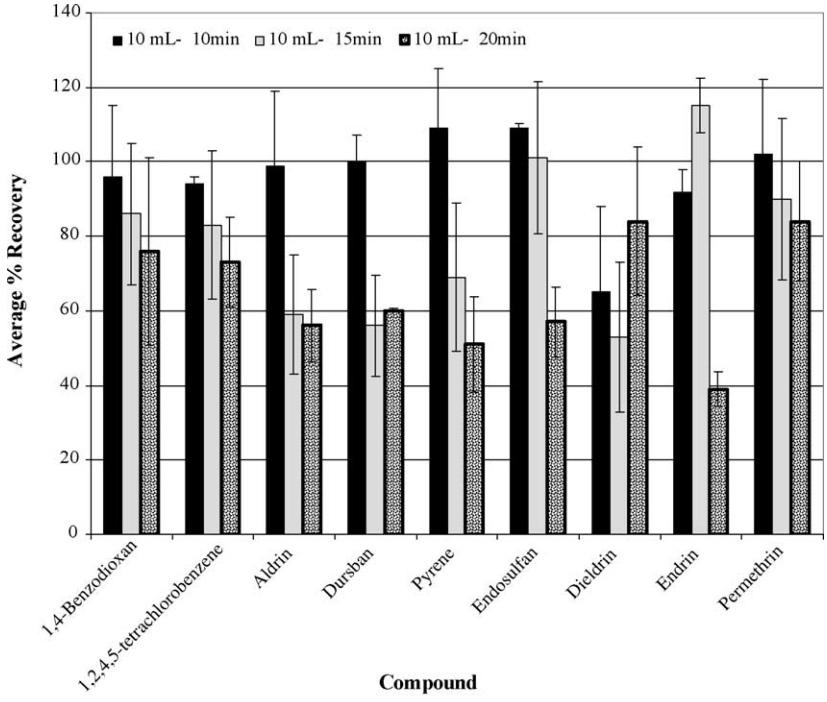

Fig. 3. Effect of extraction time in the MAE. The extraction conditions were as follow 10 mL acetone–hexane (2:1), and 60 °C.

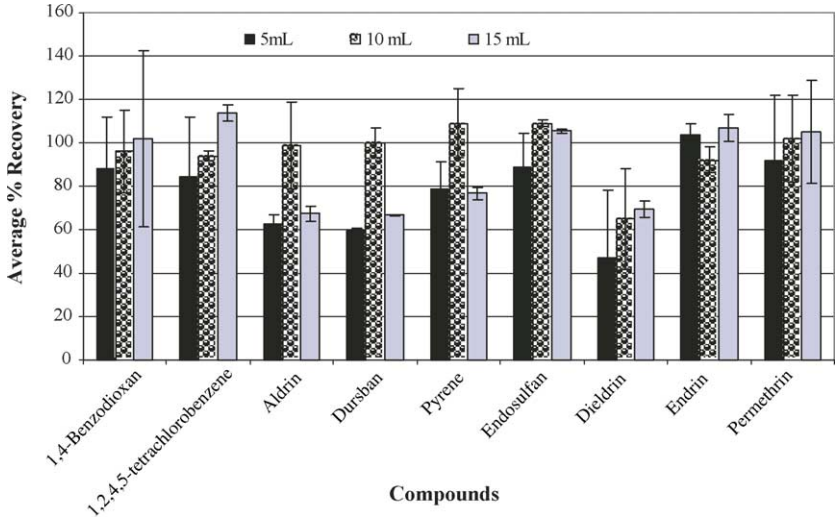

Fig. 4. Effect of extraction volume in MAE (extraction conditions were: 60 °C, 15 min, and solvent acetone–hexane (2:1)).

(polypropylene) and analytes associate with this material. The temperature program of ten min of extraction was chosen as the optimum program for our purpose.

Then three volumes of extraction were tested (5 mL, 10 mL and 15 mL). Fig. 4 shows the results of this experiment. An increase in the extraction volume resulted in an increase in the percents of recovery for some compounds and in decreases for others. A volume of 10 mL was chosen as the optimum volume because overall better reproducibility was seen and good recoveries were obtained. However, 15 mL could also be used in most cases.

3.2. SPME optimization

Prior to the development of SPME procedures for the determination of organic pollutant residues in real sebum samples, it is necessary to optimize the SPME experimental parameters, through the use of model matrix samples spiked with standards at a concentration of 25 ppb. In order to determine the optimum SPME coating type for the broad range of compound characteristics in this study, initial experiments were focused on the selection of the fiber coating. Seven commercially available fiber types were evaluated for the extraction of the representative standards from the model matrix. The PDMS 100 μm and 7 μm, PDMS/DVB 65 μm, PA 85 μm, 50/30 μm PDMS/DVB/CAR μm, 75 μm CAR/PDMS, ND 70 μm CW/DVB fibers were chosen for this study. Fig. 5 shows the comparison of the extraction efficiency of the different fibers. PDMS is a better coating for pyrene, dursban, aldrin and dieldrin but it fails to extract 1,4-benzodioxan sufficiently well. PDMS/DVB/CAR was chosen for optimization of the procedure since it can extract all the analytes with acceptable sensitivity.

The time to reach equilibrium in solid phase microextraction is influenced by the effectiveness of sample stirring. Magnetic stirring has been mainly applied for SPME in biomedi-

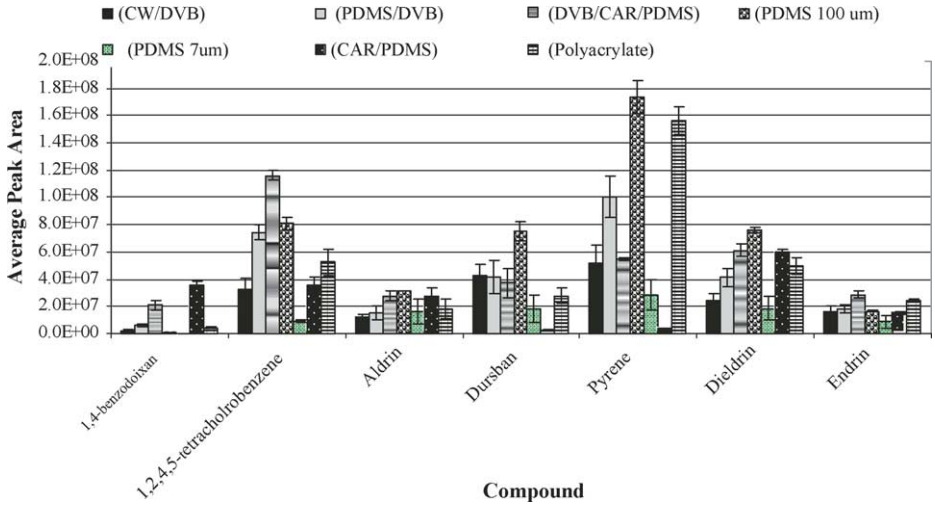

Fig. 5. Effect of fiber coating in the extraction of the standards spiked in the model matrix. Absorption time: 30 min, stirring rate: 700 rpm, desorption time 15 min, Temperature: 260 °C.

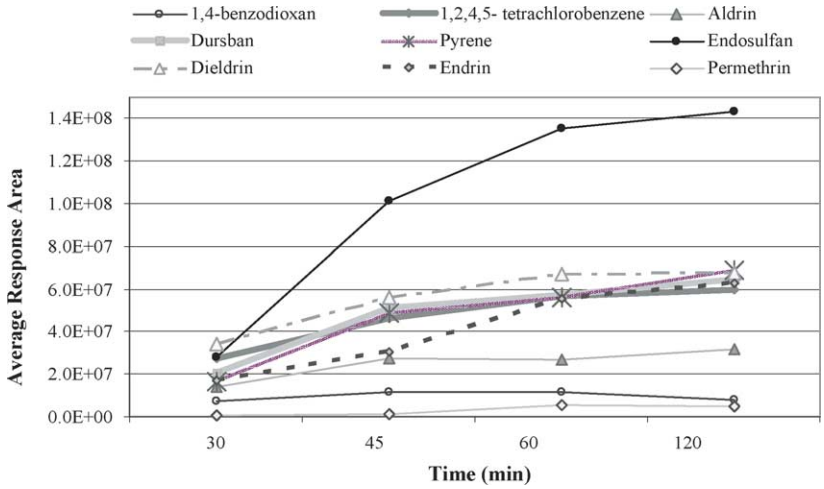

Fig. 6. Extraction time profile for the standards studied in the model matrix. Fiber type: PDMS/DVB 30/50 um, stirring rate: 1000 rpm, desorption time: 15 min, temperature: 40 °C.

cal analysis [24]. The stirring rate effect was evaluated in the range of 500–1000 rpm. In general, increasing extraction efficiency was observed as the stirring rate increased. A rate of 1000 rpm was chosen as the optimum stirring rate. To determine the effect of extraction time, a profile was obtained in a range of 30–120 min. Fresh model samples with standards at 25 ppb were used for each extraction time tested. The extraction time should be the time when no further increase of peak areas is detected with increased time of extraction [22]. At an extraction time of 120 min distribution equilibrium was still not reached for some of the standards. More extraction time is required in order to reach equilibrium. An increase of time also increases the number of sebum components absorbed on the fiber (e.g. fatty acid, hydrocarbons). Fig. 6 shows the SPME time profile for each compound. Results demonstrated that at 45 min a good detection capability was seen. Although at 45 min the equilibrium is not reached, it was chosen as the extraction time for practical reasons.

Increases in extraction temperature causes an increase in extraction rate, but simultaneously a decrease in the distribution constant [22]. Different temperatures in the range of 30–80 °C were tested. Fig. 7 shows the effect of temperature in the extraction. An increase in temperature resulted in a peak area increase for some compounds and a decrease for others. It was also noticed that with the higher temperatures, 60–80 °C, less reproducibility was obtained and more sebum components were detected. An extraction temperature of 40 °C was chosen as a compromise for the mixture used.

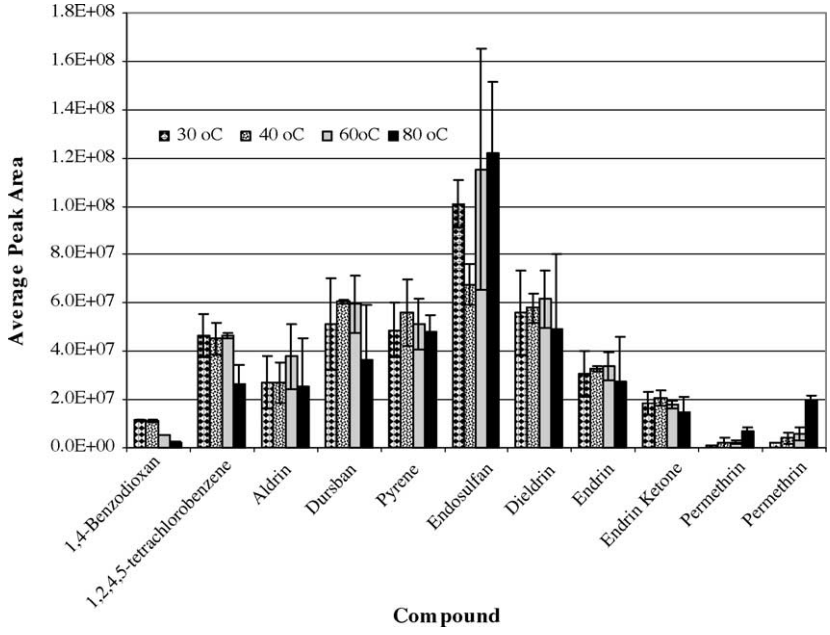

Fig. 7. Effect of temperature in the extraction of standards with SPME. Fiber type DVB/CAR/PDMS 50/30 μm, extraction time: 45 min, agitation rate 1000 rpm, desorption time 15 min, temperature range 30–40 °C.

Once the optimum extraction conditions were established (PDMS/DVB/CAR fiber, 45 min of extraction, 1000 rpm stirring rate and a extraction temperature of 40 °C), validation of the procedure was carried out as regards to linearity range, precision and estimated limit of detection for the model matrix samples. The linearity of method was evaluated through the use of calibration curves. The SPME procedures showed a linear behavior between 0.7 ppb and 25 ppb for most of the representative standards, leading to regression coefficients R^2 between 0.9994 and 0.9957 for the organochloride compounds, 0.9837 for the organophosphate pesticide (Dursban), 0.9939 for 1,4-benzodioxan and 0.9919 for pyrene. Permethrin showed linearity in a concentration range of 1–25 ppb with a R^2 of 0.9045. The precision of the method was evaluated under optimum conditions, by performing three extractions from the diluted sebum model matrix which contained all the standards studied at a concentration of 25 ppb. The precision expressed as the relative standard deviation (%R.S.D.) of the SPME extraction ranged between 1% and 23% for 1,4-benzodioxan and dursban, respectively.

Detection limits will vary with the affinity of the analyte for the PDMS/DVB/CAR fiber coating. The detection limits were estimated based on signal-to-noise S/N (higher than three times the noise). For endosulfan, that was the most responsive standard, with a S/N of 119 at 0.7 ppb; theoretically a S/N of 3 would be obtained at 18 ppt. For Permethrin, the least responsive standard, a S/N of 10 was obtained at 1 ppb; a detection limit (S/N of 3) is expected to be at 300 ppt.

3.3. Application of MAE/SPME method to sebum samples

As a preliminary test, MAE/SPME/GC–MS procedures developed in the present study were applied to some sebum samples obtained from volunteers (auto body shop employees, university students). The aim of this study was to identify possible organic pollutants reflected in their sebum.

3.3.1. Collection of sebum

Sebum was collected from the forehead of each subject with Sebutape patches (Cu Derm Corp., Dallas, TX). Before application the foreheads were cleansed thoroughly with isopropyl alcohol swabs and allowed to dry for approximately 1 min. Sebutapes were applied to the forehead with tweezers. Sebum was collected for 45 min intervals in a room at 25 °C. During the sampling period additional sebutapes were exposed to the same environment for use it as blanks. After the collection period, patches were removed and stored in glass petri dishes sealed in plastic in bags at −30 °C until analysis.

3.3.2. MAE-SPME–GC/MS analysis

Sebutapes were extracted by placing patches in the microwave extraction vessels, adding 10 mL of acetone:hexane (2:1) and using a temperature program in which the first 5 min the temperature increased from 25 °C to 60 °C and then the temperature was held at 60 °C for 5 min. After the extraction the vessels were cooled down to room temperature before opening. The extracts were transferred to mL tubes for pre-concentration. The extracts were pre-concentrated with an inert gas (N_2) to 1 mL. During the pre-concentration to 1 mL the walls of the tube were rinsed with acetone. An aliquot of 0.25 mL was diluted to 5 mL using nanopure water and extracted with the SPME method optimized for study.

A broad range of exogenous compounds was detected in the samples. The identifications were done by the NIST/EPA/NIH Mass Spectral library. Only correlations above 80% were considered. In addition a visual comparison was performed of the spectra and the best fit from the library. The method was able to detect families of compounds with different functionalities and polarities. Components of the sebum and skin surface lipids were detected. In addition exogenous compounds were detected. Some PAHs are identified (e.g. naphthalene, 1-methylnaphthalene and 1,6-methylnaphthalene). Compounds like phthalates, parabens and ingredients of pesticides (e.g. diethyltolueneamide) among others were detected. The chromatograms of a real sebum sample and sebutape blank sample are shown in Fig. 8. Table 1 lists the exogenous compounds that have been detected in this sample.

From the toxicological point of view of the compounds detected azulene, naphthalene, the methylnaphthalenes and diethyltoluamide (DEET) are seen as well as the estrogenic methylparaben propylparaben and the phthalates. However, the toxicological interest is in the potential of the method to detect a broad range of types of compounds of high toxicological significance as demonstrated by the diversity of standards seen in the method development and the list in Table 1. Most of the methods reported in the literature are target methods, specific to a family of compounds [3,14,25]. Our method could be used to detect more than one family of compounds in the same analysis.

The compounds azulene, naphthalene and methylnaphthalene are polyaromatic hydrocarbons, compounds that are known to have carcinogenic properties [2]. Phthalate esters are one of the families of compounds used as plastic additives. Many of these compounds or their metabolites (mono phthalates) are known to have toxic effects to the endocrine system, carcinogenic properties and teratogenic effects [26–28]. As an example di(2-ethylhexyl) phthalate has proven to decrease sperm counts and decline in activity of sperm metabolic enzyme [26]. Parabens are used as preservatives in cosmetic, food and pharmaceutical products to which the human population is exposed. Recently, it was demonstrated that parabens have oestrogenic properties. Parabens have a high oil/water partition coefficient and water solubility decrease with increase in ester chain length [29]. Therefore, if any parabens do enter the human body intact, they may be able to accumulate in fatty components of body tissues in a similar manner to that of other lipophilic pollutants that are known to bioaccumulate e.g. PAHs [30]. Sebum is an oily matrix and considering the lipophilicity of these compounds, it seems

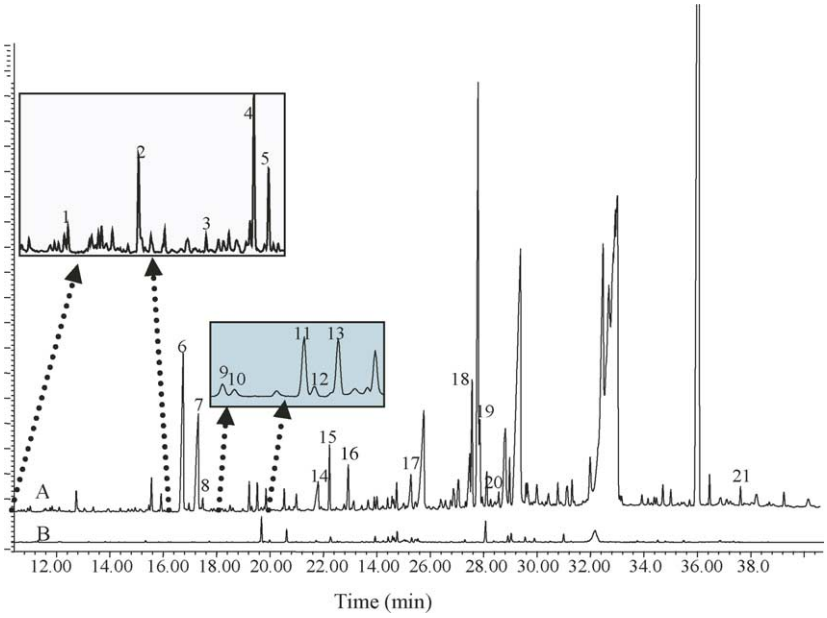

Fig. 8. (A) Chromatogram of a real sebum sample; (B) chromatogram of a sebutape blank.

likely that their secretion in sebum could represent an important route for their elimination from the body.

3.4. Comparison with other methods

An attractive aspect of this method is the relatively low amount of organic solvent that is used. The other method reported by Kimiyoshi uses 30 mL of acetone/hexane (2:1, v/v) [3]. Ours has less than half. In other representative methods in which sebum is analyzed for cocaine and codeine volumes, such as 3 mL of hexane followed by 5 mL of phosphate buffer are used [14]. For the analysis of sebum components, such as fatty acids, sterol and squalene 400–700 mL chloroform–methanol or hexane are used [25].

Table 1
List of some of the exogenous compounds detected in one of the samples

Peak number[a]	Compound	RT (min)	Correlation[b]	ng/mL[c]
1	Durol	11.1	943	5
2	Azulene	12.8	968	30
3	4,7-Dimethylindan	14.5	912	2
4	2-Methylnaphthalene	15.7	969	39
5	1-Methylnaphthalene	16.0	936	21
6	2-(2-Hydroxy-2-propyl)-5-methyl-cyclohexanol	16.8	928	NQ
7	2-(1-Hydroxy-1-methylethyl)-5-methylcyclohexanol	17.4	900	NQ
8	Menthoglycol	17.6	844	NQ
9	1,3-Dimethylnaphthalene	18.6	938	10
10	1,6-Dimethylnaphthalene	18.7	897	7
11	Geranylacetone	19.3	945	NQ
12	Methylparaben	19.4	871	8
13	2,6-Di-*tert*-butylbenzoquinone	19.6	912	NQ
14	Diethyltoluamide	22.0	922	NQ
15	Diethyl phthalate	22.3	959	102
16	Propylparaben	23.0	951	87
17	Hexyl cinnamic aldehyde	25.4	927	33
18	Benzyl-*o*-hydroxybenzoate	27.7	946	NQ
19	2-Morpholinomethyl-1,3-diphenyl-2-propanol	28.0	862	NQ
20	*p*-[2-(Dimethylamino)ethyl]phenol	28.4	820	NQ
21	Mono(2-ethylhexyl) phthalate	37.7	946	49

Chromatogram of the sample is shown in Fig. 8. Only peaks of some toxicological interest are listed. The majority of the non-numbered peaks are components of sebum. Experimental conditions: MAE (10 mL acetone–hexane (2:1), 10 min of extraction; temperature: 5 min, 25–60 °C; 5 min, 60 °C); SPME: 45 min of extraction, 40 °C, stirring rate 1000 rpm. Abbreviations: RT, retention time.

[a] The peak number corresponds to the number in chromatogram A (Fig. 8).

[b] Only one correlation is shown, in most of the cases the second correlation is the same.

[c] Estimated concentration: NQ, non quantitated.

This method has a clear advantage over other methods in time since 10 samples are extracted simultaneously in 10 min. There is a 20-min cooling period in addition to a 45-min SPME concentration. This is still much less than hours or overnight periods in which patches are left soaking in solvent in another analysis [14,25,31] and other method in which after the extraction with solvents the extract is passed through a SPE columns process that require more solvent and time [3,25].

However, the more striking advantage that is achieved is the much lower detection limits obtained of the SPME pre concentration. In our approach ppt levels are seen without the use of high resolution MS and deuterated standards.

4. Conclusion

The method developed has proven successful for evaluating exposure to a broad range of compounds as evidenced by the results. This new tool opens another door to multiple applications in forensic, pharmacological, metabolism, bioaccumulation studies and more. Excellent sensitivities in the low ten of ppt are expected in the total abundance mode of detection for some compounds. Even lower limits in the range of 10^2–10^3 improvement should be possible in the selective modes of detection. Further optimization of the method will be performed. In the near future the limits of quantization will also be determined. In addition sebum samples from persons that have exposure to persistent organic pollutants will be studied (e.g. agricultural workers, pest control employees, persons living near incinerators or thermoelectric plants).

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