Talanta 134 (2015) 560–567

Contents lists available at ScienceDirect

Talanta

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

Determination of phenolic compounds in air by using cyclodextrin-silica hybrid microporous composite samplers

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ARTICLE INFO

Article history:
Received 3 August 2014
Received in revised form
17 November 2014
Accepted 22 November 2014
Available online 2 December 2014

Keywords:
Phenols
Air sampling
Air fresheners
Cyclodextrin-silica samplers
Occupational exposure

ABSTRACT

An analytical method for the determination of phenolic compounds in air samples based on the use of cyclodextrin-silica hybrid microporous composite samplers is proposed. The method allows the determination of phenol, guaiacol, cresol isomers, eugenol, 4-ethylphenol and 4-ethylguaiacol in workplaces according to the Norm UNE-EN 1076:2009 for active sampling. Therefore, the proposed method offers an alternative for the assessment of the occupational exposure to phenol and cresol isomers. The detection limits of the proposed method are lower than those for the NIOSH Method 2546. Storage time of samples almost reaches 44 days. Recovery values for phenol, guaiacol, o-cresol, m-cresol, p-cresol, 4-ethylguaiacol, eugenol and 4-ethylphenol are 109%, 99%, 102%, 94%, 94%, 91%, 95% and 102%, respectively with a coefficient of variation below 6%. The method has been applied to the assessment of exposure in different areas of a farm and regarding the quantification of these compounds in the vapors generated by burning incense sticks and an essential oil marketed as air fresheners. The acquired results are comparable with those provided from a reference method for a 95% of confidence level. The possible use of these samplers for the sampling of other toxic compounds such as phthalates is evaluated by qualitative analysis of extracts from incense sticks and essential oil samples.

1. Introduction

Most of the generally accepted classifications of VOCs are based on their physicochemical properties. The World Health Organization (WHO) suggested that the term "volatile organic compounds" should cover only compounds adsorbed on a solid sorbent and whose boiling points lie between 50 °C and 260 °C. By contrast, the US Environmental Protection Agency (EPA) definition of VOCs includes polar and non-polar C2–C10 compounds, whose vapor pressure at 25 °C exceeds 13.33 Pa. Moreover, VOCs can be classified in accordance with a number of their properties as degree of volatility, ozone-forming potential, polarity or their effects on particular ecosystems [1].

The evaluation of VOCs in ambient and workplace air requires the use of a sampling technique to take a representative sample and avoid any variation in their composition. Since the concentration of contaminants varies over time, small sample volumes are not considered representative samples and accordingly short sampling times are not recommended [2]. Moreover, the low levels of pollutants in air samples make enrichment to be necessary. This enrichment is determined by detector sensitivity and quantification requirements [3]. The principal techniques for sampling analytes from atmospheric air that combine the isolation of analytes and their enrichment are: dynamic techniques, passive techniques and denuder techniques [4].

The principal disadvantages of passive techniques are that the enrichment factor is dependent on ambient conditions and also that it is less effective than other sampling techniques. Denuder techniques require laminar flow through the tube and denuder preparation is time-consuming and laborious [4]. The collection of samples using dynamic techniques has a high cost but, on the other side, it encloses a very effective enrichment. Then, the use of solid adsorbents and active sampling is usually recommended to evaluate workplace exposure. [3,5,6].

The use of solid adsorbents requires the optimization of retention and desorption conditions as well as the determination of the recovery percentage. Norm UNE-EN 1076:2009 describes the requirements and test methods for measuring gases and vapors using pumped [7]. This norm indicates that the recovery can be obtained by using standard gaseous mixtures or from spiked sampling tubes.

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http://dx.doi.org/10.1016/j.talanta.2014.11.057
0039-9140/

Several techniques to prepare standard gaseous mixtures have been proposed, which can be classified in static techniques, dynamic techniques and mixed techniques [8,9].

VOCs include aliphatic and aromatic hydrocarbons, aldehydes, ketones, ethers, acids, alcohols or phenolic compounds as phenol and alkylphenols. Specifically focusing on the phenols, their emission in the atmosphere is due to emissions of some plant species, animal, snuff smoke and even the use of air fresheners and cleaning products, in addition to industrial activities [10–15].

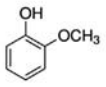
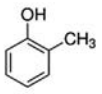
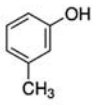
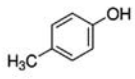
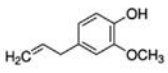
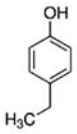
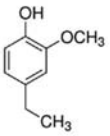
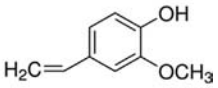
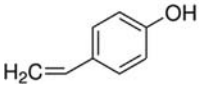
Intensive agricultural activities can be a major source of pollution and bad odors for the environment, so these facilities should be located in areas away from the population. For example, the literature describes the presence of high levels of mercaptans, phenol, xylene, 2-methyl-1-propanol, toluene, 4-ethylphenol, … in the indoor and outdoor air of cattle and pig fattening farms [11,12].

Volatile phenols and other VOCs are found in indoor air and they are originated from various sources such as the use of air fresheners (incense or essential oils) or cigarette smoke. In this regard, high levels of phenol, cresol, toluene or xylene have been found in rooms perfumed with incense [14,15], as well as in places where tobacco smoke tends to accumulate [13].

A traditional method for sampling phenols is related to their retention as phenolates by using impingers containing an aqueous solution of sodium hydroxide. However, it usually requires the preconcentration of the analytes prior to their quantification [16–18]. Alternatively, solid adsorbents can be used, being silica gel followed by solvent desorption the most common. The polarity of the adsorbed compound determines the binding strength of the silica gel; high-polarity compounds will displace low-polarity compounds. The tendency of silica gel to adsorb water vapor and displace collected components is its chief disadvantage [2]. The use of thermal desorption and other sorbents as porous polymeric sorbents is also described [19,20].

On the other hand, the use of solid phase microextraction (SPME) has been proposed for the sampling of phenols in air samples. The SPME has proven to be a very useful tool for situations in which the analyte concentration could be considered as nearly constant in time. The use of SPME has been proposed for

Table 1
Boiling point and structure of some volatile phenols [29].

Compound		CAS	$T_{boil.}$
Phenol		108-95-2	181.8 °C
2-Methoxyphenol (Guaiacol)		90-05-1	205.0 °C
2-Methylphenol (o-Cresol)		95-48-7	191.0 °C
3-Methylphenol (m-Cresol)		108-39-4	202.3 °C
4-Methylphenol (p-Cresol)		106-44-5	202 °C
2-Methoxy-4-(2-propenyl)phenol (Eugenol)		97-53-0	255 °C
4-Ethylphenol		123-07-9	219 °C
4-Ethyl-2-methoxyphenol(4-Ethylguaiacol)		2785-89-9	246.5 °C
2-Methoxy-4-vinylphenol		7786-61-0	245 °C
4-Vinylphenol		2628-17-3	206.2 °C

occupational exposure assessment and for indoor air quality control [20–22].

Recently we have designed solid phase-based samplers containing immobilized CDs for determining VOCs in air samples [23,24]. These samplers provide quantitative recoveries of BTEX and the evaluation of these compounds in workplaces has been reported.

The literature describes the formation of inclusion complexes between cyclodextrins and some phenols. Divakar et al. study the interaction of guaiacol and eugenol with cyclodextrins. These molecules exhibit identical orientations with the phenyl ring within the cyclodextrin cavity and the hydroxyl and methoxyl groups projected outside [25,26].

The formation of inclusion complexes with cyclodextrins are used to increase molecular stability. Cyclodextrins stabilize volatile compounds and, if the active centers of the molecule are included in the torus of the cyclodextrins, they decrease their reactivity thus their stability is increased. For example, eugenol (component of vegetable-based essential oils) is not stable when exposed to air, light or heat, and can be stabilized by the formation of inclusion complexes with cyclodextrins [27].

Liquid and gas chromatography are the analytical techniques used to carry out the determination of phenols. Reversed-phase high-performance liquid chromatography has been mainly used for the determination of phenols in different samples. Chiral stationary phases such as cyclodextrins allow the separation of cresol isomers [28]. Gas chromatography implies a derivatization step and/ or the use of a sensitive and selective detector as the electron capture detector because the use of FID detectors does not provide enough sensitivity. Moreover, the determination of phenols in a complex matrix implies the use of selective detectors such as mass spectrometry because the retention time is not sufficient for the compound identification prior to quantification.

The aim of this work is to study the possible use of cyclodextrin-silica hybrid microporous composite for the sampling of volatile phenols in air samples. Several volatile alkylphenols have been selected to carry out this study, namely: phenol, guaiacol, cresol isomers, eugenol, 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol and 4-vinyl, 2-methoxyphenol (Table 1). The Spanish occupational safety and health agency suggests that the acceptable levels of phenol and cresols isomers are 8 mg m^{-3} and 22 mg m^{-3}, respectively [30].

2. Material and methods

2.1. Reagents

The following reagents were used: Methanol, acetone and acetonitrile HPLC grade Scharlau (Barcelona, Spain), acetic acid and sodium hydroxide Panreac (Montcada i Reixac, Spain), Tetraethylortosilicate, α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) Fluka (Buchs, Switzerland), methyl-β-cyclodextrin (β-MCD) and 2-hydroxipropyl-β-cyclodextrin (β-HPCD) Aldrich (St. Louis, MO).

Stock standards in methanol were prepared from phenol (Ph), o-cresol (o-C), m-cresol (m-C), p-cresol (p-C) and guaiacol (G) (Fluka) and from 4-ethylguaiacol (4-EG), eugenol (E), 4-ethylphenol (4-EPh), 4-vinylphenol (4-VPh) and 2-methoxy-4-vinylphenol (2-M-4VPh) (Aldrich). Solutions were kept at 4 °C.

Saturated alkane standard solution of 1000 μg mL^{-1} in hexane was used to calculate Kovats index.

Sampling was carried out by using solid phases based on cyclodextrin-silica hybrid microporous nanocomposites. The nano-micrometric organization of these solid phases consists in well dispersed and accessible cyclodextrins trapped in the interconnected cage-like micropore system of a silica xerogel. The general formula of these solids is $(CD)_xSiO_{1.5}(OH)_{0.5}.0.7H_2O$ and details on the synthesis procedure and characterization have been described previously in detail [24].

In the present work β-$CD_{0.0007}SiO_{1.5}(OH)_{0.5}.0.7H_2O$ was selected as solid phase based on the recovery of phenols and the solubility of the cyclodextrin in the desorption solvent. The porosity and easy accessibility to CD molecules was supported by textural parameters such as total area (352.2 m^2/g), pore volume (0.16 cm^3/g) and pore size (1.18 nm). Moreover, NMR data confirm that the β–CD structure is preserved and does not undergo degradation under the preparative and working conditions.

Once the β-$CD_{0.0007}SiO_{1.5}(OH)_{0.5}$ $0.7H_2O$ solid phase was synthesized according to the general preparative strategy [24], sampling tubes were prepared using glass tubes with an internal diameter of 4 mm and a length of 80 mm. The glass tubes contain two sections separated by a solid phase porous medium. The front part contains twice of solid phase than the rear section. In particular, sampling tubes were prepared containing 500 and 250 mg of hybrid material (with a grain size in the 600–1000 μm range in order to avoid excessive particle packing) in the front and rear sections, respectively.

Sampling tubes of silica type NIOSH (140 mg/70 mg) were obtained from Dräger (Lübeck, Germany).

2.2. Instrumental analysis

2.2.1. HPLC-fluorescence detection analysis

Separation of phenols was achieved using an L-7100 liquid chromatograph from Merck–Hitachi (Darmstadt, Germany) equipped with an F-1080 fluorescence detector (Merck–Hitachi), an L-2300 column oven (Hitachi, Tokyo, Japan) and an L-7614 degasser (VWR International, Darmstadt, Germany). Detection was carried out by using an excitation and emission program. Analytical column (25 cm × 4.6 mm I.D., 5 μm particle size) and guard column (2 cm × 4 mm I.D., 5 μm particle size) were β-cyclodextrin bonded phases Astec CYCLOBOND I 2000, both from Supelco (Bellefonte, PA, USA). The temperature of the column and the guard column were thermostatically controlled. Sample injection volume and flow rate employed were 2 μL and 1 mL/min, respectively. The resolution factor (R_s), linearity and detection limit were calculated following IUPAC recommendations [31].

The literature indicates that the separation mechanism in reverse phase mode employing a β-CD stationary phase is mainly due to inclusion complexation. The pH of the aqueous mobile phases promotes the formation of hydrogen bonds between the stationary phase and the polar groups of the analytes, so by decreasing the pH, lower retention times are obtained. The concentration of the buffer solution also affects the resolution of the chromatographic peaks, decreasing the resolution when increasing buffer concentration. Moreover, methanol provides better resolution than acetonitrile phases because the former has the weakest solvent interaction with the cyclodextrin cavity. Operation at low temperatures favours the separation, but increases the analysis time and system pressure [32].

2.2.2. GC–MS analysis

A Thermo (Austin, TX, USA) Focus GC system coupled with a mass detector DSQII and equipped with a AI3000 autosampler was used to obtain analytical signals of sample analysis. A stationary phase HP-5MS (5% phenyl methylpolysiloxane) 30 m/0.25 mm/0.25 μm was used to carry out the separation. Helium was used as the carrier gas at a constant flow of 0.7 mL min^{-1}. The temperature was initially set at 40 °C (2 min) and then heated to 280 °C at 5 °C min^{-1}. The mass selective detector (MSD) was operated in electron impact mode with a potential ionization of 70 eV and a source temperature of 250 °C. The scan range used in SCAN mode was m/z 40–340, whereas ions m/z 94, 107, 120, 124, 137, 150, 164 for phenol, cresol isomers and 4-ethylphenol, 4-vinylphenol, guaiacol,

4-ethylguaiacol, 2-methoxy-4-vinylphenol and eugenol, respectively, were selected in SIM mode. The interface temperature and the injector temperature were set at 250 °C. 1 μL of the sample was injected in splitless mode with a solvent delay of 2 min [14].

2.3. Sample collection

Air samples were collected using a portable Buck-Genie VSS-5 pump from A.P. Buck (Orlando, FL, USA), previously calibrated with a Multicon KS external flow calibrator (Dräger, Lübeck, Germany). The samples were collected using the above described sampling tubes mounted in the connecting tube of the pump. After collection, the tubes were closed with plastic caps, sealed in plastic bags and stored at 4 °C.

To collect the environmental samples the sample flow rate was set at 110 mL $min^{-1}$and samples were collected for 3 h.

For the sampling of vapors generated by incense sticks, the system shown in Fig. 1 was used. The system comprises a holder to secure the incense and a bell with a hole that allows sample collection. The bell is placed on 0.5 cm supports to enable the entry of air and thus enabling combustion. During sampling the solid phase is colored brown. The sampling rate was set at 200 mL min^{-1} and the sample was collected while the incense was still burning, plus after an additional 5 min in order to sample the generated vapors. An essential oil sample was also collected using this system. For this purpose, 500 μL of essential oil were placed in the container shown in the Fig. 1 which was heated with a candle to make the oil evaporate. The sampling time was enough for the sample to evaporate and an additional 5 min were needed to complete the sampling of the generated vapors.

2.4. Optimization study of retention and desorption conditions.

The optimization study was carried out using spiked sampling tubes prepared according to the UNE-EN 1076:2009 recommended procedure for active sampling tubes [7]. For this purpose, 10 μL of multicomponent stock solution of phenols containing 2 μg of each compound were injected directly onto the sorbent. Subsequently, the tubes were allowed to equilibrate in air for several minutes, the ends of each tube were capped and the tubes were allowed to stand overnight at 4 °C. The blank was prepared in a similar manner.

Desorption was carried out independently in the two sorbent sections. To do this, two sections were placed in separated extraction tubes and, after extraction, were filtered and injected in the chromatographic system. Recovery was calculated as a ratio of the obtained and added amount of each compound.

The extraction procedure was optimized by varying one parameter at a time, while keeping the others constant. The parameters studied were the nature and amount of the solid phase, nature and volume of the solvent and the time and temperature of desorption. Furthermore, the influence of the amount of contaminants and sample storage time were studied.

Fig. 1. Manifold employed for the sampling.

2.5. Analytical figures of merit

The reproducibility study has been carried out based on the triplicate recoveries obtained for each one of the polluting agents tested using solid phases of β-CD. To that end, 10 μL of the multicomponent solution in acetonitrile were injected directly onto the front sorbent section. Subsequently, the tubes were allowed to equilibrate in air for several minutes, the ends of each tube were capped and the tubes were allowed to stand overnight at 4 °C. The blank was prepared similarly. Desorption was carried out under previously optimized conditions.

The detection and quantification limits of the method were calculated considering a volume of air sample of 40 l.

2.6. Analysis of samples

The proposed procedure (Fig. 2) was applied to the occupational exposure of phenols in air samples collected at a farm house, a block and a chicken coop. In addition, samples of vapors generated by lavender, rosemary, sandalwood, apple and pine sticks and vapors of pine essential oil were collected. Moreover samples of sticks were collected by using tubes of silica type NIOSH and analyzed by reference method carrying out desorption with 1 ml of acetone and stirred occasionally for 30 min [33].

In all cases a blank was prepared using a sampler tube without passing air through it.

Air samples were analyzed by HPLC. The other samples, due to the complexity of the chromatograms, were analyzed by GC/MS. In this case, before the quantification of phenols, the peaks from their mass spectra were identified by using bibliographic data and the calculated retention index was compared with that indicated in the literature. Furthermore, the compounds were identified presenting a report percentage above 0.05%. In order to calculate the retention index, a solution containing n-alkanes was injected by using the same temperature program.

3. Results and discussion

3.1. HPLC fluorescence detection

Preliminary studies were carried out in isocratic and gradient elution conditions with binary mobile phase methanol/acetic acid solution 0.1 M and acetonitrile/acetic acid solution 0.1 M comprising between 85% and 97% for organic solvent. The fluorimetric detection was carried out at 275 nm/300 nm.

The use of acetonitrile and gradient elution decreases the time of analysis without affecting the resolution of the peaks. Best results were obtained working with the following gradient program:

- $t=0$ min 100% acetic acid solution
- $t=5$ min 98% acetic acid solution and 2% acetonitrile (for 9 min)
- $t=15$ min 90% acetic acid solution and 10% acetonitrile (for 10 min)

The pH and buffer concentration affects the resolution and lower temperatures generally lead to increased retention and resolution when β-CD bonded phases are used to carry out the separation [32]. Subsequently, the influence of pH, acetate/acetic acid buffer concentration and temperature were studied. For this purpose, the temperature varied between 10 °C and 40 °C, buffer solutions of pH 4, 4.5 and 5.0 were used and their concentration varied between 0.005 and 0.05 M. The retention times corresponding to phenol, guaiacol, o-cresol and 4-vinylphenol were the most highly affected. Overlaps are noted between phenol and guaiacol, o-cresol and guaiacol and between eugenol and 4-vinylphenol for buffer concentrations higher

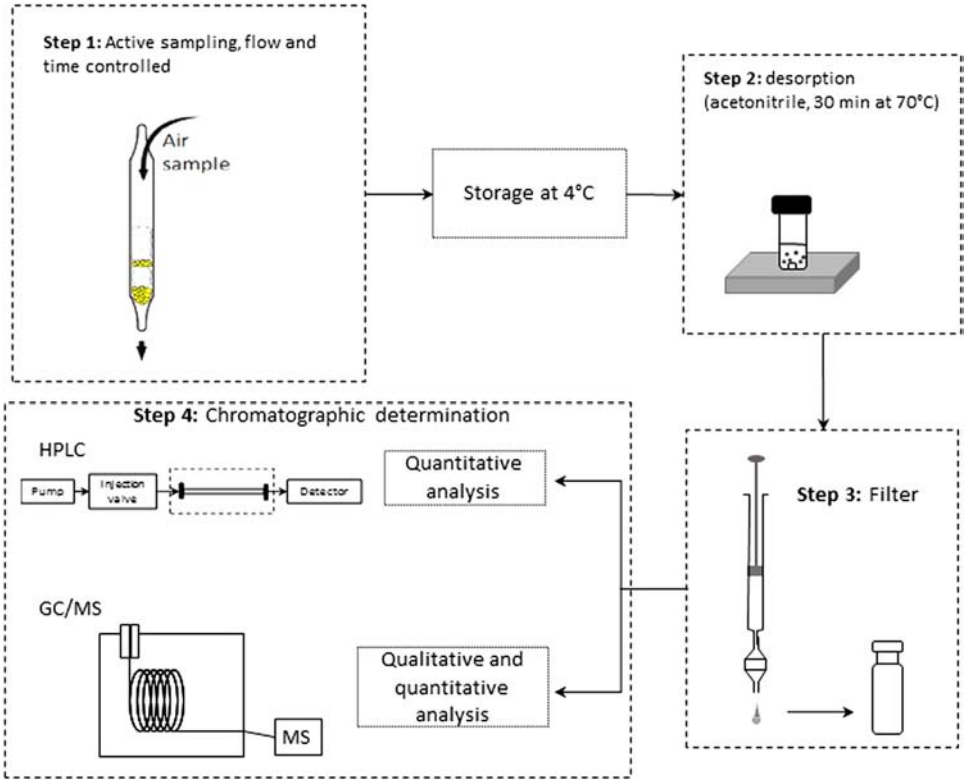


Fig. 2. Recommended procedure.

than 0.01 M. The best results are achieved at temperatures between 20 °C and 30 °C with a buffer concentration of 0.005 M and pH 4.

Once separation conditions had been optimized, excitation and emission spectra of the different compounds were obtained in order to locate emission and excitation maximums and to propose a wavelength program allowing detection of compounds with the highest sensitivity (Table 2).

Based on these results, a complete separation of all studied compounds is possible. Table 3 shows the sensitivity, linearity and resolution for the studied phenols.

3.2. Retention and desorption

The study of the nature of the solid phases was carried out based on α-CD, β-CD, γ-CD, β-MCD and β-HPCD sampling tubes. HPLC- fluorescence detection was used for the optimization study.

Since the presence of cyclodextrin in the solution can affect the fluorescence signal, calibration solutions were prepared in the presence of blank extracts obtained from the treatment of solid phases at 55 °C for 30 min with methanol, ethanol, acetonitrile and sodium hydroxide solution 0.01 M. In the case of extracts obtained from alkali, before their injection in the chromatographic system, solutions were neutralized with acetic acid to pH 4.5.

The results indicate that the fluorescence signal of 2-M-4-VPh, 4-VPh, o-C, E and G are strongly affected in alkali extracts. The variation of the fluorescence is lower when the extracts are obtained with acetonitrile. This effect is greater for the more soluble cyclodextrins. Specifically, changes in the fluorescence signal are greater when using solid phases α-CD (more soluble cyclodextrin) and lower when working with solid phase β-CD (less soluble cyclodextrin).

Specifically, the fluorescence signal varies about 30% for 2-M-4-VPh and 4-VPh in presence of blank extracts obtained from solid phase β-CD and acetonitrile as a solvent, and 5% for all other phenols. Therefore, to perform the quantification of the analytes, calibration solutions must be prepared from blank extracts obtained under the same conditions as the samples. The selected phase to carry out the rest of the study was β-CD and acetonitrile was used as desorption solvent.

Table 2
Maxima wavelengths and proposed program of wavelengths.

Compound	λ_{ex}(nm)	λ_{em}(nm)	**Wavelength program**	
			Time	λ_{ex}–λ_{em}
Ph	272	298	0–9.2 min	280–310 nm
G	275	312		
o-C	272	302		
m-C	275	301		
2-M-4-VPh	266/300	339	9.2–11.0 min	290–320 nm
p-C	277	306		
4-EG	279	319	11–17 min	270–320 nm
E	279	314		
4-VPh	264	333		
4-EPh	278	307	17–25 min	280–310 nm

Table 3
Analytical figures of merit of HPLC fluorescence detection.

Compound	Sensitivity (L mg^{-1})	LOD (mg L^{-1})	Linearity limit (mg L^{-1})	Resolution
Ph	47,600 ± 1600	0.07	> 8	–
G	78,000 ± 2000	0.04	> 9	1.34
o-C	64,500 ± 700	0.05	> 9	1.24
m-C	96,000 ± 1300	0.04	> 8	3.83
2-M-4-VPh	100,700 ± 700	0.03	> 8	7.07
p-C	34,300 ± 200	0.10	> 9	3.58
4-EG	26,800 ± 200	0.13	> 8	3.45
E	20,700 ± 300	0.17	> 8	3.93
4-VPh	4800 ± 130	0.7	> 20	1.21
4-EPh	54,400 ± 700	0.06	> 9	5.56

The influence of the solvent volume, temperature and time desorption on recovery was studied. To do so, the procedure outlined above was followed and 10 μL of a multicomponent solution containing 2 μg of each contaminant were injected. Desorption was carried out using a water bath and a magnetic stirrer. The desorption was carried out by using 1, 2 and 3 mL of acetonitrile, at 55 °C, 65 °C and 75 °C and for 15, 30 and 45 min.

Recovery increased between 55 °C and 65 °C remaining constant from 65 °C to 75 °C. Moreover, recovery increased when heating time increases remained constant after 30 min. Therefore, a 30 min stirring time and a controlling temperature between 65 °C and 75 °C were selected. 2 mL of acetonitrile was selected as optimal to carry out the desorption providing enhanced sensitivity and recovery.

The reproducibility study was based on the triplicate recoveries obtained for each of the pollutants following the recommended procedure. Moreover, the extracts were analyzed by GC/MS in order to confirm if the low recovery of 4-VPh and 2-M-4-VPh is due to an uncorrected matrix effect or to the non-retention and / or non-desorption of the analytes.

Determination by GC/MS confirmed the low recovery of 4-HPV and 2-M-4-HPV (36% and 33%, respectively). Accordingly, these results suggest the non-retention and/or desorption of these compounds in the solid phase. Results for Ph, G, o-C, m-C, p-C, 4-EG, E and 4-EPh (Table 4) indicate enhanced reproducibility with a coefficient of variation below 6%. Moreover, recoveries obtained were higher than 90%.

From the elemental analysis performed, it was concluded that in 500 mg of solid phase 5.8 10^{-6} mol of β-CD are linked. So, the influence of the amount of contaminant was deducted by injecting amounts above the stoichiometric ratio pollutant:β-CD. To that end, 10 μL of multicomponent solutions containing between 2×10^{-7} and 2×10^{-6} mol of phenolic compounds were injected. The recoveries differ in less than 10% from the tabulated values in all cases.

Finally, desorption carried out after 44 days of sample storage shows no significant differences in recovery. Therefore, the storage time is at least 44 days.

A diagram of the proposed process is illustrated in Fig. 2 and the detection and quantification limits are shown in Table 4.

Compared to other methods described in the literature (Table 5), the proposed method provides similar recoveries, reproducibility and time analysis to those of other methods and assesses the occupational exposure of phenol and cresols isomers according to UNE-EN 1076 [7]. Detection limits of the proposed method are lower than NIOSH Method 2546 being therefore more sensitive. Storage time is almost 44 days. On the other hand, the method provides recoveries higher than 75% and standard deviations lower than 10% for eugenol, guaiacol, 4-ethylguaiacol and 4-ethylphenol determination. Therefore, the method is suitable for the assessment of occupational exposure in accordance with UNE-EN 1076. Furthermore, the method offers an improved reproducibility compared to methods based on thermal desorption. Detection limits of guaiacol, eugenol and cresol isomers are lower than detection limits provided by other proposed methods.

Table 4
Analytical figures of merit of proposed method.

Compound	Recovery (%)	RSD (%)	LOD (mg m^{-3})		LOQ (mg m^{-3})	
			HPLC-Fl	GC/MS	HPLC-Fl	GC/MS
Ph	109	4	0.004	0.02	0.012	0.06
G	99	4	0.002	0.008	0.006	0.02
o-C	102	3	0.003	0.03	0.009	0.09
m-C	94	3	0.002	0.017	0.006	0.05
p-C	94	6	0.005	0.017	0.015	0.05
4-EG	91	4	0.007	0.004	0.02	0.012
E	95	0.8	0.009	0.004	0.03	0.012
4-EPh	102	2	0.003	0.03	0.009	0.08

3.3. Sample analysis

The procedure was applied to the determination of phenols in air samples collected at a farm house, a stable and a chicken coop. Moreover, gases generated from the combustion of air fresheners, such as lavender sticks, rosemary sticks, pine sticks, sandalwood sticks and apple sticks were analyzed. Finally, the procedure was applied to the determination of vapors generated from pine essential oil. Occupational exposure was evaluated based on the results obtained for air samples by comparing them with the tabulated values of TLV-TWA [30].

The use of retention time to perform the identification of compounds from a chromatogram is not suitable in samples with a complex matrix. However, the chromatogram obtained by GC/MS can be used to perform the identification and the quantification of phenols. Accordingly, samples of incense sticks and essential oil were analyzed by GC/MS and HPLC with fluorescence detection. The other samples were only analyzed by HPLC.

To collect the air samples from the farm house, the stable and the chicken coop the sample flow rate was set at 110 mL min^{-1} and the sampling time was set at three hours. Table 6 shows the results obtained. The chromatograms from HPLC solely indicate the presence m-cresol, while the rest of phenols were below the detection limit. Furthermore, the chromatograms show no presence of other compounds in the samples. The concentration of m-cresol was of the order of 0.1 mg m^{-3} so, assuming that the concentration remains constant over time, in all cases the concentration was below the TLV- TWA (22 mg m^{-3}), resulting in an exposure index (the ratio between daily exposure and TLV-TWA) lower than 0.1 in all cases.

To collect samples of gases and vapors generated by fresheners, the sampling system shown in Fig. 1 was used. The sample flow rate was adjusted to 200 mL min^{-1} and the sample was collected while the incense was burning and after an additional 5 min. The essential oil sample was collected by the evaporation of 500 μL into the sampling system. All samples were collected by triplicate. After desorption, samples were analyzed by HPLC and by GC.

The chromatograms obtained from HPLC show the possible presence of phenols in the samples. However, the complexity of chromatograms renders the identification and quantification of analytes impossible.

Therefore, the identification of compounds present in the samples was performed based on their mass spectra and by comparison with the retention indices reported in the literature [34,35] (see supporting information table S1) working with a temperature ramp and with a stationary phase HP-5MS (5% phenyl methylpolysiloxane) 30 m/0.25 mm/0.25 μm or similar. Only compounds whose relative abundance (RA) was higher than 0.05% were considered. Finally, once identified, phenols were quantified (Table 7).

Many of the compounds identified are terpenes, compounds that form part of essential oils such as linalool, borneol, camphor or eucalyptol. Some studies related contact dermatitis [36] with some typical compounds in fragrances such as cinnamaldehyde in the lavander sample, eugenol in lavander, rosemary, pine and sandalwood samples and ϒ-Methylionone in sandalwood and oil samples. Linalool was only detected in lavander sample, bomeol was detected in pine and oil samples and camphor and eukalyptol were only detected in rosemary samples. None of the terpenes were detected in apple samples.

On the other hand, diethyl phthalate was detected in all samples. Other phthalates were identified, such as dibutyl phthalate in samples of rosemary and sandalwood sticks and bis(2-methoxyethyl) phthalate and diisobutyl phthalate in rosemary

Table 5
Comparison of proposed and other published methods.

Sampling	Analytes	Recovery	RSD	Storage time	LOD	Time consuming	Reference
Impingers	Ph, o-C, m-C, p-C	> 80%	< 20%	48 h	1–5 mL m^{-3}	Few min	16
Impingers + HP-SPME	Ph, G, o-C, m-C, p-C, E	> 80%	7–18%	No data	3.5–10 μg m^{-3}	> 90 min	17
Silica sampling tubes	Ph, o-C, m-C, p-C	82.5–88.8%	2.4–6.2%	48 h	no data	120 min	18
XAD-7 sampling tubes	Ph, o-C, m-C, p-C	> 90%	2.8%	Almost 30 days	1–3 μg per sample	30 min	19
Tenax TA sampling tubes	p-C, 4-EPh	95–1–128%	9.3–22.2%	Almost 120 h	No data	8 min	20
Cyclodextrin-silica hybrid microporous composite samplers	Ph, G, o-C, m-C, p-C, 4-EG, E, 4-EPh	91–109%	0.8–6%	Almost 44 days	LOD[a]: 4–30 μg m^{-3} LOD[b]: 1.7–8 μg m^{-3} LOD[c]: 0.04–0.4 μg/sample LOD[d]: 0.07–0.3 μg/sample	30 min	Proposed method

[a] Calculated considering a sample volume of 40 l and GC/MS detection.
[b] Calculated considering a sample volume of 40 l and HPLC-fluorimetric detection.
[c] GC/MS detection.
[d] HPLC-fluorimetric detection

Table 6
Results of air samples.

Sample	m-cresol (μg m^{-3})	*I*
Farm house	8.7	3.9×10^{-4}
Stable	13.5	6.1×10^{-4}
Chicken coop	12.4	5.6×10^{-4}

sticks. Phthalates provide flexibility and durability to plastic and are also used as solvents. Phthalates are commonly present in cosmetics, perfumes, toys, fresheners…. These compounds can affect the liver, kidneys, lungs and the hormonal and reproductive systems, primarily the testes of male babies [37]. As can be seen, not any other phthalate compound was detected in the apple sample, excluding diethyl phthalate as said before. Phthalates are found in the list of regulated chemicals, while some chemicals such as diisobutyl phthalate and dibutyl phthalate are included in the list of chemicals for which use restrictions are proposed based on their adverse health effects. Specifically, dibutyl phthalate has already been banned in the manufacture of toys due to its effect on male babies [38].

As shown in Table S1, phenol and guaiacol were detected in lavander, rosemary, pine, sandalwood and apple samples whereas 4-ethylguaiacol and eugenol were detected in lavander, rosemary, pine and sandalwood samples, excluding the apple samples. p-Cresol and/or m-cresol were found in lavander, pine and sandalwood samples highlighting that o-cresol was the only one detected in the lavander sample. 4-ethylphenol was not detected in none of the samples. Table 7 shows the quantification results of these compounds.

As noted, the content per stick ranges varies from 0.07 to 1.49 mg m^{-3}. As can be seen in this table, all samples contain phenol and guaiacol concentrations above the quantification limit and in none of them has the presence of 4-ethylphenol been detected. Regarding o-cresol, m-cresol and p-cresol isomers, only the quantification of the meta and para isomers in sandalwood and pine samples. Finally, lavender and apple samplers have 4-ethylguaiacol concentration under the quantification and detection limit and in none of the apple samplers has the presence of eugenol been detected.

As indicated previously, incense sticks samples were also analyzed after the retention of the analytes using silica tube samples and desorption with 1 mL of acetone during 30 s and shaking occasionally. Furthermore, in order to know the environmental concentration, the recovery of the studied compounds has been calculated as in the case of proposed tube samplers, because the recovery may vary with the type and batch of the samplers utilized [33]. The obtained recovery was 91%, 85%, 81%, 75%, 85%, 73% and 55% to phenol, o-cresol, m-cresol + p-cresol, guaiacol, 4-ethylphenol, 4-ethylguaiacol and eugenol respectively. The results are shown in Table 7.

The regression analysis for the results obtained by proposed and reference method would indicate that for a 95% confidence level, the values of the slope and the intercept of this line are 1 and 0, respectively. This indicate that the proposed method does not give a constant relative error and does not require a blank correction.

As can be seen, the coefficient of variation for the triplicate analysis is higher than that which is shown in Table 4. On the other hand, the coefficient of variation of the obtained results by the proposed method and by the reference method is smaller than the obtained coefficient of variation for the triplicate analysis. This phenomenon is due to the variation between samples; the amount of incense contained in each stick is not reproducible and it cannot be weighed as when we weigh the sample, both stick and incense are being weighed without any distinction. That is to say that the obtained coefficients of variation are higher than the calculated reproducibility due to the nature of the sample itself.

Based on the carried out study, the following conclusions may be made:

Cyclodextrin-silica hybrid microporous composite samplers are suitable for the sampling of phenol, cresol isomers, eugenol, guaiacol, 4-ethylguaiacol and 4-ethylphenol in air samples.

The proposed method constitutes an alternative to other methods described in the literature for the purpose of evaluating the exposure to phenol and cresol isomers at the workplace.

The proposed method complies with the requirements established in the Norm UNE-EN 1076:2009 and thus, can be used to evaluate occupational exposures of eugenol, guaiacol, 4-ethylguaiacol and 4-ethylphenol.

In comparison with the reference method, the proposed method gives significantly higher recoveries for guaiacol and 4-ethylguaiacol and much higher for eugenol. Therefore, it results suitable for its determination in working atmospheres. It is important to mention that in the eugenol case the use of silica samplers provides a recovery of 55%, well below 75%, which does not allow its use for the assessment of exposure to this compound in working atmospheres [7]. The detection limits of the proposed method are lower

Table 7
Quantitative determination (mg m^{-3}) of phenolic compounds in samples ($n=3$) by reference (A) and proposed method (B). Results expressed as $\bar{x} \pm s$

Compound	Lavender sticks		Rosemary sticks		Pine sticks		Sandalwood sticks		Apple sticks	
	A	B	A	B	A	B	A	B	A	B
Ph	1.16 ± 0.08	1.01 ± 0.10	0.78 ± 0.07	0.78 ± 0.07	1.1 ± 0.2	1.1 ± 0.2	1.49 ± 0.08	1.3 ± 0.3	0.95 ± 0.08	1.01 ± 0.15
G	0.74 ± 0.16	0.75 ± 0.14	0.74 ± 0.06	0.9 ± 0.2	1.8 ± 0.3	1.8 ± 0.2	1.08 ± 0.16	1.03 ± 0.08	0.51 ± 0.02	0.69 ± 0.04
o-C	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< < LOQ	< LOQ	< LOD	< LOD
m-C + p-C	< LOQ	< LOQ	< LOQ	< LOQ	0.19 ± 0.10	0.43 ± 0.06	0.15 ± 0.03	0.118 ± 0.012	< LOD	< LOQ
4-EG	< LOQ	< LOD	0.047 ± 0.005	0.07 ± 0.03	0.06 ± 0.02	0.07 ± 0.02	0.12 ± 0.03	0.102 ± 0.012	< LOQ	< LOQ
E	0.8 ± 0.3	0.6 ± 0.3	0.08 ± 0.02	0.08 ± 0.04	0.215 ± 0.015	0.170 ± 0.010	0.10 ± 0.02	0.12 ± 0.02	< LOD	< LOD
4-EPh	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

than the NIOSH Method 2546 and the method is therefore more sensitive. The reproducibility of the proposed method is higher than that of methods based on thermal desorption. The qualitative analysis of the gases and vapors generated by sticks and essential oils indicate that these samplers may be used to determine terpene compounds and phthalates in air samples.

The use of sticks and essential oil as fresheners produce potentially toxic compounds. The presence of phthalates in these samples implies that baby-related use is not recommended. Furthermore, some of the identified compounds can cause dermatitis so their use is not recommended in areas where there may be particularly sensitive individuals. To conclude, the obtained results for the proposed method are coincident with those of the reference method for a confidence level of 95%.

Acknowledgments

This research was supported by the Ministerio de Economía y Competitividad, Spain, MAT2012-38429-C04-03 and the Generalitat Valenciana, Valéncia, Spain (PROMETEO/2009/108). The authors thank the institutional support from VLC/CAMPUS (MCIs Sensores and "Técnicas Analíticas Rápidas" and Medicina Regenerativa].

Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.11.057.

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