2014 5th International Conference on Environmental Science and Technology
IPCBEE vol.69 (2014) © (2014) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2014. V69. 13

Direct-Ultrasonic Assisted Microextraction Coupled with RTL-GC-FID/GC-MS as a Future Standard Procedure for Monitoring 26 Potentially Allergenic Fragrances in Water Samples

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Abstract. This research topic grew up as a result of awareness of constant anthropogenic and natural input to the environment constituents of personal care products (PCPs) residues. This group of emerging pollutants encompasses a wide range of chemicals, including potentially allergenic fragrance compounds. The aim of the present study was to investigate presence of 26 fragrance allergens in water samples. Simple and rapid methodology based on direct-ultrasonic assisted liquid-liquid microextraction (USALLME) followed by gas chromatography with flame ionization detector (GC-FID) and gas chromatography with mass spectrometry (GC-MS) using Retention Time Locking has been developed. GC-MS analyses were performed with inlet pressure adjusted at 7.29 psi to lock a retention time at 27.500 min for n-pentadecane. GC-FID analyses were performed with inlet pressure adjusted to give a retention time of 70.000 min for n-pentadecane which was set up at 38.032 psi. Finally several real water samples were investigated with the application of the proposed method.

Keywords: Ultrasonic probe, ultrasound-assisted emulsification–microextraction, isolation and preconcentration procedure, organic analytes, environmental analysis, water samples.

1. Introduction

A group of chemicals included in most personal care products are fragrances suspected to cause allergenic reactions [1]. The number of fragrance compounds employed in everyday products is very large [2] as a proof, The Scientific Committee on Consumer Products list (SCCP) of perfume and aromatic raw materials contains 2750 entries [3]. The actual number of fragrances used in hygiene and personal care products is larger, because fragrances which do not appear on the list are used in cosmetics and other products too [4]. For all of those substances, responsible or suspected to be responsible of causing adverse human health reactions the use had to be limited and/or strictly regulated [5]. For this reason The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has identified and published 26 of these ingredients as most likely to cause contact allergies [6]. It must be emphasized that, in the recent years, the risk of contact allergy, induced by perfumery ingredients has been the object of scientific debate [7], [8]. In addition to the widespread use and exposure to fragranced products many of the raw fragrance materials have limited available health and safety data.

Nowadays, in the European Union, the allergen topic is raised [9]. One of the several European regulations concerning allergens in food, cosmetics or other consumer products is “the 26 allergens rule” for fragrances - Article 1(10) in Directive 2003/15/EC, the 7th amendment of the Cosmetic Directive [10]. The EU Cosmetics Directive was replaced by the new European Cosmetics Regulation which came into force in 2012, and the European chemical regime is being changed considerably by the European Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Also the methods for evaluation and risk assessment have been improved lately [11], [12]. Taking into account the law aspect, health aspect and environmental aspect there is a need to develop appropriate methodologies for

the determination of personal care products residues. Between the methods currently available for fragrance allergens determination, those based on ultrasounds techniques have proved accurate and reliable [13], [14].

In the present study, a new microextraction technique for aqueous samples, based on the usage of direct source of ultrasonication as a assistance force for emulsion creation of suspected allergen fragrances (as listed in the Cosmetic Directive) in water samples, was developed. This technique combines the microextraction system and ultrasonic radiation in one step and is based on the emulsification of a micro-volume of organic extractant in an aqueous sample by ultrasound radiation, and further separation of organic and inorganic liquid phases by centrifugation. Linearity and detection limits (LODs) are studied in order to assess the performance of the proposed method. Several water samples were analyzed to demonstrate the applicability of the proposed method.

In Direct-USALLME, the application of ultrasonic radiation facilitates the emulsification phenomenon and accelerates the mass-transfer process between two immiscible phases for the inside of the sample. This improves the extraction efficiency in a minimum time. Developed technique can be employed as a simple and efficient extraction and preconcentration procedure for organic compounds in aqueous samples. The main drawback of this method is lack of possibility to automate.

2. Experimental

2.1. Chemicals and Reagents

All fragrance allergen reagents and internal standard 1,2-dimethyl-3-nitrobenzene were purchased from Aldrich (Sigma– Aldrich Chemie GmbH, Poznan, Poland), Fluka (Fluka Chemie GmbH, Poznan, Poland), chloroform, methanol and sodium chloride were provided by Merck (Warsaw, Poland). All solvents and reagents were analytical grade. Individual stock solutions of each compound (at the concentration level 5 $\mu g \cdot \mu L^{-1}$) and a stock mixture solution of all targets (at the concentration level 100 $ng \cdot \mu L^{-1}$) were prepared in methanol. Further dilutions and mixtures were prepared in methanol and stored in amber vials at 4 ℃.

2.2. Water Sampling

Water samples, 1000 mL each, were collected from river (Kacza, Poland) (RW - 1 samples), water treatment plant (Gdansk, Poland) (WTP - 2 sample), sewage treatment plant (Gdansk, Poland) (STP - 6 sample). All samples were stored in amber glass bottles closed with cork (without a headspace gas phase under the cork) at 4 ℃, protected from light. In the all of those samples the 26 suspected allergen fragrances are expected.

2.3. Sample Treatment

Before further steps of analytical procedure all water samples were filtrated through a 0.45 μm Millipore HA membrane filter (Filtrak Brandt GmbH Wiesenbad - Plattenthal, Thermalbad Wiesenbad, Germany). The excess of free chlorine was removed directly after the sampling by addition of sodium thiosulphate (0.1 $mg \cdot mL^{-1}$) to the total sample volume.

2.4. Extraction Procedure: Direct-USALLME

Aliquots of 7 mL sample were placed in 9 mL conical-bottom glass centrifuge tubes with teflon caps. 300 mg of NaCl were added for salting out the sample. The addition of salt decreases the solubility of analytes in the aqueous phase and promote the transfer of analytes towards the organic phase [13]. The next step of sample procedure was the addition of 100 μL chloroform (extractant solvent) and 20 μL of a 0,45 μg mL-1 of 1,2-dimethyl-3-nitrobenzene in methanol as internal standard. In the tube was then immersed a 7 mm of diameter titanium sonotrode (Ultrasonic probe Hielsher UP200Ht, Germany) in such a way that the end of the sonotrode was approximately 5 mm below the sample surface. Extractions were performed at puls mode of sonification, with energy limit of 400 W s and amplitude of 75%. During the sonification the sample was cooled down to avoid possible derivatization and/or volatilization of the analytes. As a result, oil-in-water (O/W) emulsions of extractant (dispersed phase) in water (continuous phase) were formed. Emulsions were then disrupted by centrifugation at 3400 rpm for 8 min and the organic phase was sedimented at the bottom of the conical tube. Extractant phase was removed using a 100 μL Hamilton syringe (J.S. Hamilton Poland S.A., Gdynia, Poland) and transferred to a 50 μL glass insert located in a 1.8 mL gas chromatography vial.

2.5. Gas Chromatographic Analysis

In flavor and fragrance quality control, retention indices are still frequently used as a complementary technique to gas chromatography. Several libraries are available with retention indices for many fragrance compounds. Retention indices are less dependent on operational parameters than absolute retention times, but they still depending significantly on the column type (stationary phase and supplier), on the temperature program, and to a lesser extent, on the carrier gas velocity. Therefore it is sometimes difficult to reproduce published retention indices in different laboratories. Developments in GC have led to the ability of locking and matching retention times. Using retention time locking, it is no longer necessary to calculate the retention index, but the absolute retention time can be used as an identification tool. Retention times are still dependent on operating conditions, but small differences in carrier gas velocity and column length are compensated by re-locking the GC method by adjustment of the column head-pressure [15].

In this work the retention time was re-locked for n-pentadecane, at 70 min in GC-FID analysis and at 35 min in GC-MS analysis.

The gas chromatography detailed parameters and conditions are shown in Table 1.

Table 1: Gas chromatography parameters and conditions

Gas chromatography conditions		
Detector	Flame Ionization Detector (280 ℃)	Mass Spectrometer (transfer line 300 ℃ ion source 230 ℃ quadrupole 150 ℃)
Column	DB-5ms 60m x 250 µm x 1 µm	DB-5ms 30m x 0,25mm x 0,25 µm
Injection (Temperature)	Splitless (250 ℃)	Split (250 ℃), split ratio 5:1
Injection volume	1 µl	1 µl
Carrier gas	Helium	Helium
Pressure	38,032 psi	7,29 psi
GC method	Retention Time Locking (for pentadecane)	
Oven program	50 ℃ -> 2 ℃/min -> 280 ℃ (115 min analysis time)	60 ℃ -> 3 ℃/min -> 280 ℃ (76 min analysis time)
Software	Agilent ChemStation	MSD ChemStation
Fragrance data base	Flavor2.scd Flavors RTL Database by Agilent	Adams EO library Mass Spectral Library, 2205 cpds. and Flavor2.L Flavors RTL Database by Agilent

3. Results and Discussion

3.1. Optimization of Microextraction Process

Regarding the direct-ultrasonic assisted liquid-liquid microextraction process the selection of an appropriate extraction parameters such as mode of sonification, introduced ultrasonic energy and volume of the extraction solvent is an important issue. Corresponding optimization parameters were studied as follows:

- volume of organic solvent - three organic solvent volumes, 100 µL, 200 µL and 300 µL, were tested in the experimental design in an attempt to achieve the highest extraction efficiency for the target compounds;
- mode of sonification - the effect of this factor was evaluated at two levels - constant mode and pulse mode of sonification;
- energy limit - the effect of this factor was examined from 200 to 400 W s.

In view of the results of the optimization study, the experimental conditions selected for the for the simultaneous microextraction of the target compounds from water samples were as follows: 100 µL as extractant solvent volume, mode of sonification - pulse, energy limit - 400 W s. Emulsification was observed in every case.

3.2. Quality Control and Quality Assurance

After the target analytes were identified and chromatographically separated, selected validation parameters were studied which is confirming the reliability of the developed method for the analysis of 26 suspected allergens in water samples. Linear range and limits of detection (LODs) were defined to validate the proposed method. The calibrations were performed using standard solutions of 26 suspected allergen fragrances prepared with two concentration ranges: low concentration range of 0.5–10 ng $\cdot \mu L^{-1}$ and high concentration range of 10–300 ng $\cdot \mu L^{-1}$.The evaluation is made by software adjustment of the best linear

tendency (regression) between the peak area and analyte concentration. The calibration curves were linear in the studied range with coefficient of determination R^2 ranged from 0.979 to 0.999.

Method limit of detection (calculated with the method based on a signal-to-noise ratio of 3 (S/N=3) for the sample volume of 7 mL and 100 µl of organic solvent used for the analyte extraction) lies between 7.14 mg Ł$^{-1}$ (for α-pinene) and 100 mg Ł$^{-1}$ (for farnesol).The sensitivity of the proposed method can be considered satisfactory for the target compounds.

3.3. Environmental Sample Analysis

Finally, the proposed method was applied for the determination of fragrance allergens in several non-spiked water samples, including river water, water treatment plant water and sewage treatment plant water. Concentration of 26 suspected fragrance allergens in water samples are given in Table 2. Up of 17 out of 26 allergen fragrances was confirmed in the 9 analyzed water samples. The average number of these compounds ranged from 5 to 7, while two different samples contained none of the studied allergen fragrances. The most common allergen fragrance detected in nearly all samples is linalool; at concentration ranging from 2.7 to 170 mg· L^{-1}.

The least common detected compounds are: citral (detected only in one sewage treatment plant sample at concentration level of 90 mg· L^{-1}) and benzyl cinnamate (detected only in one sewage treatment plant sample at concentration level of 15 mg· L^{-1}). The highest found concentration correspond to farnesol (250 mg· L^{-1}) in sewage treatment plant sample and the lowest one correspond to limonene (0.87 mg· L^{-1}) in river water sample.

Table 2: Concentration of 26 suspected fragrance allergens in water samples.

Compound	Sample type / analytes concentration in environmental waters calculated on their concentration in the extract converting to concentration in whole sample (mg Ł$^{-1}$)								
	RW	STP 1 rs II	STP 1 rs I	STP 1 tw	STP 1 rs I	STP 1 rs II	STP 1 tw	WTP bt	WTP at
alpha-Pinene	<LOD	13	1,4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Limonene	**0,87**	13	1,1	<LOD	<LOD	1,4	<LOD	<LOD	<LOD
Linalool	2,7	170	64	39	24	44	9,3	<LOD	<LOD
Benzyl alcohol	19	34	21	<LOD	21	23	<LOD	<LOD	<LOD
Citronellol	<LOD	90	41	<LOD	37	41	<LOD	<LOD	<LOD
Citral	<LOD	90	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Eugenol	<LOD	69	40	<LOD	20	23	<LOD	<LOD	<LOD
Methyl eugenol	<LOD	54	21	<LOD	26	27	<LOD	<LOD	<LOD
Lilial	<LOD	9,9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Lyral	<LOD	24	<LOD	<LOD	19	17	<LOD	<LOD	<LOD
Coumarin	<LOD	46	23	<LOD	16	17	<LOD	<LOD	<LOD
Amyl cinnamic alcohol	<LOD	30	<LOD	<LOD	21	24	<LOD	<LOD	<LOD
Amylcinnamaldehyde	<LOD	6,6	27	<LOD	19	24	<LOD	<LOD	<LOD
Farnesol	<LOD	170	9,9	120	**250**	140	<LOD	<LOD	<LOD
Benzyl benzoate	9,7	9,4	<LOD	<LOD	<LOD	16	<LOD	<LOD	<LOD
Benzyl salicylate	<LOD	90	4,9	<LOD	4,3	7,7	190	<LOD	<LOD
Benzyl cinnamate	<LOD	15	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

<LOD – value below limit of detection for specific compound
RW – river water
STP 1 rs – sewage treatment plant water 1, raw sewage
STP 1 tw - sewage treatment plant water 1, treated wastewater
STP 2 rs - sewage treatment plant water 2, raw sewage
STP 2 tw - sewage treatment plant water 2, treated wastewater
WTP bt - water treatment plant - before treatment
WTP at - water treatment plant - after treatment

highest found concentration
lowest found concentration
most common fragrance allergen
least common fragrance allergen

4. Conclusion

The method based on the Direct-USALLME coupled to GC-FID and GC-MS developed for the analysis of 26 regulated allergen fragrances in water has proved to be an efficient, simple, rapid, environmentally friendly microextraction technique. Under the optimized conditions the method limits of detection were on the level of 7.14 mg Ł$^{-1}$ (the lowest) and 100 mg Ł$^{-1}$ (the highest) which satisfies many needs. The proposed method was applied to the analysis of 9 real water samples including river samples, sewage treatment plant samples, water treatment plant samples. The presence of allergen fragrances was confirmed in 7 samples, demonstrating the ubiquity of the target compounds.

5. Acknowledgements

Scientific supervision support from Professor Jacek Namiesnik is gratefully acknowledged.

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