

Gas Chromatography Analysis with Olfactometric Detection (GC-O): an Innovative Approach for Chemical Characterization of Odor Active Volatile Organic Compounds (VOCs) Emitted from a Consumer Product

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The gas chromatography-olfactometry (GC-O) methodology couples traditional gas chromatographic analysis with sensory detection in order to identify odor active compounds in complex mixtures. Volatile Organic Compounds (VOCs) emitted from materials and consumer products can affect indoor air quality (IAQ) in terms of odor annoyance and other adverse effects on human health. Hence, the main purpose of the present paper was to recognize the odor active VOCs emitted by a specific consumer product and responsible of the odor annoyance perceived, performing head-space experiments followed by GC/MS-O analysis both at ambient temperature and during heating (condition of use). Ten odor active VOCs belonging to different chemical classes were detected; Aromatic Hydrocarbons (Benzene, Toluene, m/p-Xylene), Phenols (Phenol), Aldehydes (Benzaldehyde, Nonanal), Chlorinated Aromatic Hydrocarbons (Chlorobenzene, 1,4-Dichlorobenzene), Polycyclic Aromatic Hydrocarbons (Naphthalene) and Anhydrides (Phthalic Anhydride). Naphthalene was recognized as the organic compound that mainly contributed to the overall odor perceived. Moreover, it was observed that the product's heating resulted in an increasing of VOCs emission and in odor perception. Experimental data obtained highlighted the potentialities of GC/MS-O methodological approach to identify off-flavors deriving from materials and to provide useful indications to manufacturers to improve the acceptability of odorous emissions.

1. Introduction

In the recent years, research activities have been carried out in several scientific fields to recognize odor active compounds and to investigate human odor perception with the aim of developing direct applications in industry. Nowadays research activities focus the attention on improving the quality of scented products, such as perfumes and food, through the selection of natural or synthetic odorous compounds in order to make them more pleasing and desirable for end-users (Schilling et al., 2010; Yeomans, 2013). Other scientific studies are interested in the evaluation of the impact of odorous compounds on the quality of indoor air, human life and environment. Odor active compounds may derive from industrial activities (landfills, wastewater treatment plants, refineries, tanneries) or may be emitted from most commonly used building and packaging materials (Knudsen et al., 2007; Beghi et al., 2012; González et al., 2013). The main purpose of research activity is to recognize odor active compounds and to relate qualitative and quantitative information with human perception. Instrumental approaches based on Gas Chromatography coupled with Mass Spectrometry (GC/MS) have been widely developed (Davoli et al., 2003; Dincer et al., 2006) but the main limitation is related to the complexity of the odor mixture and to the lack of information regarding human perception; so a correlation between identity and concentration of odor active compounds and the associated olfactory stimulus can not be found. As the human olfactory system is

recognized as the most sensitive odor detector, a remarkable improvement in odor assessment is gained by coupling GC/MS analysis with olfactometric detection (GC-MS/O) (Brattoli et al., 2013). GC-MS/O methodology allows to establish a correlation between the chemical nature and concentration of specific odor compounds with the human perception of smell. Odor active compounds are detected by properly trained human assessors that sniff the GC effluent, putting their nose at a specifically designed sniffing port (ODP) connected in parallel to conventional detectors. They evaluate the duration of the odor stimulus (from start to end) providing a qualitative description of the odor perceived and give information about its intensity. Nowadays GC-MS/O methodology is widely applied in several environmental fields and has proved to be a valuable method for the selection and identification of odor active compounds from a complex mixture as the human nose is a much more sensitive detector than the conventional ones (flame ionization detector, mass spectrometer) for many volatile chemicals that are often present at concentrations lower than the instrumental detection limit (Di Francesco et al., 2001). More specifically, GC/MS-O methodology can be a useful and reliable tool for the detection and identification of odor active Volatile Organic Compounds (VOCs) responsible for off-flavors coming from a wide range of indoor materials and consumer products. It is well known from scientific findings that VOCs emission from building materials, furniture and furnishings can deteriorate the indoor air quality (IAQ) both in terms of odor annoyance and other adverse effects on human health (Dambrosio et al., 2013). Hence the growing concern for human exposure to VOCs emitted from materials has led to the development of labeling schemes in several European Countries with the aim of reducing indoor emissions and preserving human health (ECA, 2010). GC/MS-O is primarily applied in the material industry with the purpose to control and reformulate the manufacturing processes and to give useful instruction to manufacturers to improve the acceptability of odorous emissions from any kind of products. The Directive for Registration, Evaluation, Authorization and Restriction of Chemicals, adopted by the European Union in December 2006, also focused the attention on VOCs emissions from articles (Regulation (EC) No 1907/2006). Great attention has been paid to the "non-intentional" release of chemicals from articles, registered in reasonably foreseeable conditions of use. At this regard, the primary goal of the present work is the application of GC/MS-O methodology in order to recognize the odor active VOCs responsible of the odor annoyance deriving from a specific consumer product.

2. Methods and materials

2.1 Investigated product

The object of the investigation was a portable warming bag electrically supplied, having an external coverage made of Polyethylene and manufactured in an extra-European country. Its function is to provide human health comfort, to relieve stress and to reduce muscle and joint pain. The attention was focused on this kind of consumer product as it was rejected by end-users due to the odor annoyance, especially during the heating phase, therefore this evidence was highlighted to competent authorities.

2.2 Screening olfactometric analysis

The primary goal of this work was to identify the odor active VOCs emitted by the article and responsible of the odor annoyance perceived by end-users. Previous to the identification, a screening olfactometric analysis was carried out in order to verify the objective perception of the odor nuisance. The official methodology for odor emissions assessment is the Dynamic Olfactometry, a sensorial technique standardized by the European technical laws that allows to measure the odor concentration of the overall mixture, expressed in odor unit per cubic meters (ou_E/m^3), without any information regarding its chemical composition (CEN EN 13725/2003). It is based on the use of a dilution instrument, the olfactometer, that presents the odor sample diluted with odor-free air at precise ratios to a panel of trained human assessors selected according to their perception threshold for a reference gas (n-butanol). Generally, in the first presentation the odor mixture is diluted with a very large volume of odor-free air in order to be undetectable by the human nose. In subsequent presentations, the volume of diluent is decreased by a predetermined and constant factor. In this work sensory measurements were performed by means of an olfactometer (ECOMA) equipped with four sniffing ports and set up to carry out dilutions ranging from 2^2 to 2^{16} with a constant factor equal to 2. Four assessors sniffed at a single port and communicated if an odor was detected or not (yes/no method). The sampling strategy to collect odor samples was chosen in order to simulate the real odor exposure of the end-users. Air samples were drawn by means of a depression pump placed close to the emitting source and conveyed inside a Nalophan bag, both at ambient temperature and during the article's heating phase. As the sampling took place on the surface of the bag, a contribution of the background ambient air to the odour can be excluded.

2.3 Gaschromatographic-Olfactometric analysis (GC/MS-O)

A deeper comprehension of the odor mixture composition was possible carrying out GC/MS-O analysis. The electric warming bag was introduced inside a Nalophan bag and dynamic head-space experiments were carried out. Gas-phase VOCs were conveyed by a continuous and constant ultrapure air flow and sampled on $\frac{1}{4}$ in. stainless steel tube containing Carbograph™ 4 adsorbent cartridges (35-50 mesh) by means of flow-controlled pump (Pocket Pump). Best sampling conditions were selected, according to the suggestions listed in the International Standard (ISO 16000-6:2004). The sampling flow rate was equal to 50 ml min^{-1} and the resulting sample volume collected onto the cartridge was equal to 5 Liters. The dynamic head-space experiments were carried out both at ambient temperature and during article's heating, as showed in Figure 1.

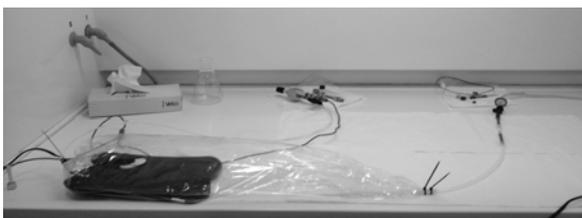


Figure 1: Dynamic head-space experiment.

The analyses were carried out using a thermal desorber (Markes International Ltd, Unity 2™) connected to a gas chromatograph (GC Agilent 7890) equipped with an olfactometric port (ODP 3 Gerstel) and connected to a mass spectrometer (MS Agilent 5975). The thermal desorption consisted of a two-stage mechanism. VOCs were desorbed from the Carbograph adsorbent cartridge at $300 \text{ }^\circ\text{C}$ and refocused onto a cold trap at $-10 \text{ }^\circ\text{C}$. Then the cold trap was flash heated to $300 \text{ }^\circ\text{C}$ and the vapors were transferred via the heated transfer line ($200 \text{ }^\circ\text{C}$) to the GC column and to the olfactometric port. The analytical column was HP5-MS ($30\text{m}\times 250\mu\text{m}\times 0.25\mu\text{m}$) and the flow of the carrier gas (Helium) was controlled by constant pressure and was equal to 1.7 ml min^{-1} . The GC oven was temperature programmed from $37 \text{ }^\circ\text{C}$ to $100 \text{ }^\circ\text{C}$ at $3.5 \text{ }^\circ\text{C min}^{-1}$, and from $100 \text{ }^\circ\text{C}$ to $250 \text{ }^\circ\text{C}$ at $15 \text{ }^\circ\text{C min}^{-1}$. The MS transfer line was 230°C and the MS was operated in electron impact (EI) ionization mode (70eV) with a mass range 20-250 m/z. Acquisition was carried out in SCAN mode. After the GC separation the column flow was splitted with the ratio 1:1, one part was led to the MS system and the other one to ODP. The effluent from the capillary column reached the olfactometric port through an uncoated transfer line (deactivated silica capillaries) and was sniffed by the assessor in a PTFE conical port, fitted to the shape of a nose. The transfer line was heated to prevent the condensation of compounds on the walls of the capillary. Auxiliary gas (make-up gas) was added to the GC effluent to prevent the drying of the assessors' nose mucous membranes, which could cause discomfort especially in longer analyses. Two trained panelists were asked to indicate when odor was perceived, pressing an electric push-button connected to the ODP, and to indicate with clear words a qualitative description of the odor. It is important to underline that, in order to obtain reproducible data, potential assessors are generally screened for sensitivity, motivation, ability to concentrate and ability to recall and recognize odor qualities (Delahunty et al., 2006). They are generally asked to refrain from smoking and eating/drinking strongly flavored foods for 1 h prior to performing GC-O analysis. They usually do not wear aftershave, perfume or strong deodorants the day of assessment (Etiévant et al., 1999; Delahunty et al., 2006). Finally, a maximum sniff time of 25–30 min is recommended since GC-O sniff duration can impact human detector performance.

3. Results and discussion

3.1 Olfactometric results

Experimental results obtained by olfactometric analysis of VOCs emission deriving from the product, both at ambient temperature and during the heating phase, are reported in Table 1. As different measurement cycles were carried out, the final odor concentrations c_{OD} were calculated as the geometric means of the values obtained for each single series and expressed in ou_E/m^3 . Moreover, taking into account that the relation between odor intensity and odor concentration is logarithmic, measure uncertainty is represented by a confidence interval not symmetric around the mean value (VDI guideline 3881,1986). Upper and lower limits of the 95 % confidence interval are also reported.

Table 1: Olfactometric results (based on three replicates)

Sample	CO _D (ou _E /m ³)	Lower limit	Upper limit	Odor description
Cold bag	31	17	73	Unpleasant
Hot bag	57	30	91	Unpleasant

The experimental data obtained reveal that an objective perception of the odor was found. Moreover, from the comparison between the cold bag and hot bag odor concentrations, 31 and 57 ou_E/m³ respectively, it is possible to observe that the heating phase determines an increasing in the odor perception. However, the difference is not very significant, even considering the uncertainty in measurement, as cold bag manifests a noticeable odor emission.

3.2 Identification of odor active VOCs by GC/MS-O

Figure 2 illustrates the GC-O TIC chromatogram related to the analysis of VOCs emissions from the investigated article at ambient temperature.

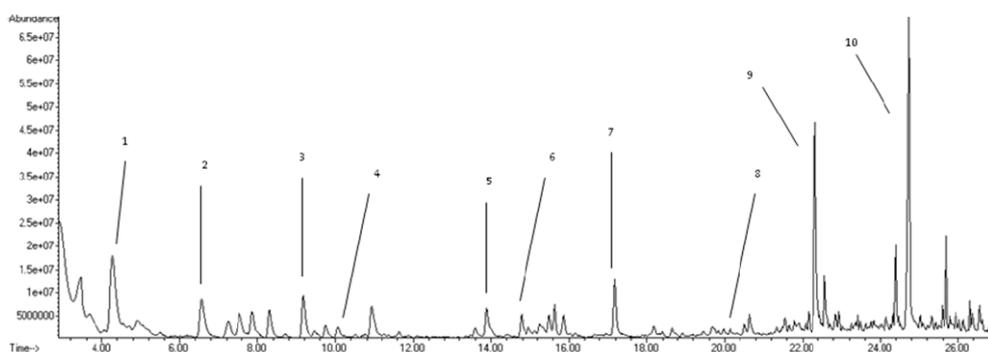


Figure 2: GC-MS chromatogram in SCAN mode related to the article's emission at ambient temperature (in table 2 are reported the identified molecules corresponding to the peak number).

Although many volatile VOCs were detected by MS, in this paper only the odor active VOCs perceived by assessors are reported. The identification of odor active VOCs was performed by comparing the mass spectra of the unknown compounds with those listed in the NIST library (Agilent Technologies). The identification was considered valid if the confidence rating of mass spectra comparison was superior or equal to 95%. The attribution was further confirmed using the retention times of authentic compounds. Identified odor active VOCs, detected at the ODP, belong to different chemical classes: Aromatic Hydrocarbons (Benzene, Toluene, m/p-Xylene), Phenols (Phenol), Aldehydes (Benzaldehyde, Nonanale), Chlorinated Aromatic Hydrocarbons (Chlorobenzene, 1,4-Dichlorobenzene), Polycyclic Aromatic Hydrocarbons (Naphthalene) and Anhydrides (Phtalic Anhydride). Many other VOCs belonging to Aliphatic and Aromatic Hydrocarbons classes were identified by GC/MS but they did not generate any sensory stimulus at the ODP. The odor active VOCs detected by the assessors sniffing at the ODP were described and qualified on the basis of an odor intensity scale, ranging from 0 (no perceptible odor) to 4 (strong odor). VOCs detected, their retention times expressed in minutes, assessors odor description and associated intensity are listed in the Table 2. Differences between the two assessors responses are specified, only when occurred (Assessor 1/2).

The odor evaluation and the simultaneous identification via GC/MS-O allowed to recognize the Naphthalene, perceived by both the assessors with an intensity level equal to 4, as the odor active compound responsible of the overall odor annoyance related to the portable warming bag.

Figure 3 shows the complex chromatogram obtained from the GC/MS-O analysis of VOCs emission when the article was turned on and heated. In this case, a greater number of odor active VOCs were detected at ODP but remained unrecognized due to the chromatogram complexity and the overlapping of co-eluting compounds resulting in a confidence rating of mass spectra comparison lower than the reference value (95%). However, it must be noticed that the increasing in temperature has a remarkable effect in odor active VOCs release. The complexity of the chromatogram, which has a great number of unresolved peaks and the large number of odorous stimuli perceived by the evaluators did not allow the identification of the compound responsible for the unpleasant smell.

Table 2: Identified GC-O detected VOCs emitted by article at ambient temperature (see figure 2 for peaks corresponding to compound number).

Compound no.	VOC	TR(min)	Odor description		Odor intensity/Score	
			Assessor 1/2	Assessor 1/2	Assessor 1/2	Assessor 1/2
1	Benzene	4.28	Solvent		2 (weak odor)	
2	Toluene	6.56	Solvent		2 (weak odor)	
3	Chlorobenzene	9.17	Resinuos		2 (weak odor)	
4	m/p-Xilene	10.06	Solvent/Sweet		3 (clear odor)	
5	Benzaldehyde	13.89	Solvent/Grass		2 (weak odor)	
6	Phenol	14.77	Mushroom		2 (weak odor)	
7	1,4-Dichlorobenzene	17.18	Solvent/Mushroom		1 (just perceptible odor)	
8	Nonanale	20.13	Unpleasant		2 (weak odor)	
9	Naphtalene	22.32	Mothball		4 (strong odor)	
10	Phtalic Anhydride	24.74	Plastic		1 (just perceptible odor)	

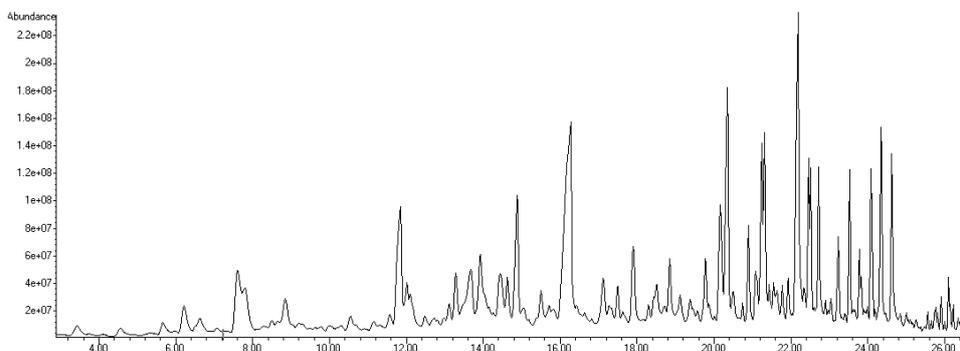


Figure 3: GC-MS chromatogram in SCAN mode related to the article's emission during heating phase.

VOCs occurrence could be explained taking into account that extra-European manufacturers could have performed specific surface treatment on the article's polymeric coverage, not only to color and stamp but also to preserve it from any kind of deterioration during long range transport (e.g. Naphtalene). Moreover it should be underlined that most of odor active VOCs detected are of particular concern from a toxicological point of view and may pose health hazard for inhalation exposure, as stated by the European CLP Regulation (Regulation (EC) No 1272/2008). Although experimental data do not allow any consideration about risk assessment, the investigation revealed the presence of Benzene recognized as a genotoxic carcinogen in humans for which no safe level of inhalation exposure can be recommended. At this regard, World Health Organization suggests to reduce or eliminate materials that release Benzene (WHO, 2010).

4. Conclusion

Characterization of odor active VOCs emitted by an electric warming bag was evaluated performing head-space experiments and applying GC/MS-O methodological approach. Ten odor active VOCs emitted by the article at ambient temperature and belonging to different chemical classes were identified: Aromatic Hydrocarbons (Benzene, Toluene, m/p-Xilene), Phenols (Phenol), Aldehydes (Benzaldehyde, Nonanale), Chlorinated Aromatic Hydrocarbons (Chlorobenzene, 1,4-Dichlorobenzene), Polycyclic Aromatic Hydrocarbons (Naphtalene) and Anhydrides (Phtalic Anhydride). The odor evaluation and the simultaneous identification via GC/MS-O allowed to recognize the Naphtalene as the organic compound responsible of the overall odor annoyance perceived. Moreover, it was observed an increasing in VOCs emission and in odor perception during the heating phase (article's condition of use). This study emphasizes the potentialities of GC/MS-O methodology and puts in evidence the importance of identifying

chemical compounds responsible for off-flavors coming from materials and consumer products in order to enhance the knowledge regarding this issue and to supply useful information to manufacturers to improve products' quality and odor acceptability.

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