Journal of Chromatography A, 1255 (2012) 163–170

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the forensic study of cadaveric volatile organic compounds released in soil by buried decaying pig carcasses

Catherine Brasseur[a], Jessica Dekeirsschieter[b], Eline M.J. Schotsmans[c], Sjaak de Koning[d], Andrew S. Wilson[c], Eric Haubruge[b], Jean-Francois Focant[a,*]

[a] CART, Organic and Biological Analytical Chemistry, Chemistry Department, University of Liège, Allée du 6 août, B6c, Sart-Tilman, B-4000 Liège, Belgium
[b] Department of Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, 2 Passage des Déportés, B-5030 Gembloux, Belgium
[c] Forensic & Archaeological Sciences, School of Life Sciences, University of Bradford, West Yorkshire BD7 1DP, United Kingdom
[d] LECO Instruments GmbH, Marie-Bernays-ring 31, 41199 Mönchengladbach, Germany

ARTICLE INFO

Article history:
Available online 3 April 2012

Keywords:
Comprehensive two-dimensional gas chromatography (GC × GC)
Time-of-flight mass spectrometry (TOFMS)
Volatile organic compound (VOC)
Cadaver decomposition
Forensic
Geotaphonomy
Scripting

ABSTRACT

This article reports on the use of comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOFMS) for forensic geotaphonomy application. Gravesoil samples were collected at various depths and analyzed for their volatile organic compound (VOC) profile. A data processing procedure was developed to highlight potential candidate marker molecules related to the decomposition process that could be isolated from the soil matrix. Some 20 specific compounds were specifically found in the soil sample taken below the carcass and 34 other compounds were found at all depths of the gravesoil samples. The group of the 20 compounds consisted of ketones, nitriles, sulfurs, heterocyclic compounds, and benzene derivatives like aldehydes, alcohols, ketones, ethers and nitriles. The group of the 34 compounds consisted of methyl-branched alkane isomers including methyl-, dimethyl-, trimethyl-, tetramethyl-, and heptamethyl-isomers ranging from C_{12} to C_{16}. A trend in the relative presence of these alkanes over the various layers of soils was observed, with an increase in the amount of the specific alkanes when coming from the carcass to the surface. Based on the specific presence of these methyl-branched alkanes in gravesoils, we created a processing method that applies a specific script to search raw data for characteristic mass spectral features related to recognizable mass fragmentation pattern. Such screening of soil samples for cadaveric decomposition signature was successfully applied on two gravesoil sites and clearly differentiates soils at proximity of buried decaying pig carcasses from control soils.

1. Introduction

Forensic geotaphonomy is a branch of forensic anthropology focusing on the geological and botanical alteration of the surrounding environment of a buried dead body. The study of biochemical changes in soil related to the grave digging, to cadaver decomposition processes and to decomposition states themselves can be used in forensic investigation to estimate post-mortem interval and possibly locate clandestine graves or hidden remains of a victim. Despite this interest, the knowledge of the relation between cadaver decomposition and soil chemistry is still limited [1]. For this reason, gravesoil is normally only used as associative evidence rather than as matrix that can potentially give information on cadaveric decomposition. It has been known for a long time that cadaver decomposition can influence gravesoil biology and chemistry [2]. Each stage of cadaver decomposition is known to have some specific effect on soil parameters (microbial activity, carbon dioxide production, pH, electrical conductivity, etc.) [3,4]. During the various decay stages of a buried cadaver, a lot of different and specific volatile organic compounds (VOCs) are also emitted [5] and released into the soil. Some of those VOCs are responsible for the typical "smell of death" that we are able to sense, although some are only detected by sensitive organisms like scavenger insects or cadaver dogs [6–8]. Several groups are studying this specific VOC emission [9–14] but most of studies concern aboveground decomposition. Additionally, despite recent advances in VOC characterization and the establishment of a decomposition odor analysis database [9], there is still a lot of work to do to better characterize the olfactive signature of a decaying body.

* Corresponding author at: University of Liège, CART, Biological and Organic Analytical Chemistry, Allée du 6 août, B6c, B-4000 Liège, Belgium.
Tel.: +32 04 366 35 31; fax: +32 04 366 43 87.
E-mail address: JF.Focant@ulg.ac.be (J.-F. Focant).

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2012.03.048

Such a characterization is essential to better understand mechanisms responsible for insect attraction and possibly be valuable for forensic entomology [15].

Analyzing mammalian decomposition products is an analytical challenge as complex reactions take place, resulting in the chemical breakdown of the body's main constituents (lipids, proteins and carbohydrates) [16]. For the analysis of VOCs produced during decomposition processes, gas chromatography (GC) coupled to mass spectrometry (MS) is the tool of choice. Solid-phase extraction (SPE) [17], solid-phase micro extraction (SPME) [9], thermal desorption (TD) [11–13,18] have been used for sample collection and transfer to GC–MS. A large range of volatile and semi-volatile low to medium polar compounds can easily be analyzed by GC–MS, although polar analytes require a derivatization step prior analysis. Because of the large number of VOCs present in the chemical profile of decomposition odor [10], GC however easily suffers from peak capacity limitations. The use of non-scanning time-of-flight (TOF) MS analyzers that offer constant ion ratio over GC peaks can resolve some of the GC co-elutions in the MS domain by spectral deconvolution. However, such analytical resolution might suffer in efficiency as many compounds are issued from the same chemical families and are therefore characterized by similar mass spectral data, which complicates the deconvolution of the MS signal. Such a complex situation is further complicated in the case of gravesoil analysis as the soil matrix itself is made of various groups of chemical components, organisms, and debris [19].

Comprehensive two-dimensional gas chromatography ($GC \times GC$) has been developed to meet an increasing need for complex sample analysis and to address limitations such as peak capacity, dynamic range and restricted specificity of one-dimensional (conventional) GC systems (1D-GC) (i.e. to improve the efficiency of the separation). $GC \times GC$ can be defined as a chromatographic technique during which a sample is subjected to two different separation processes coupled on-line [20]. In practice, the end of the first dimension (^{1}D) column is placed in a temperature controlled interface called 'the modulator' and further serially connected to the second dimension (^{2}D) column. The cryogenic modulator ensures high sampling rates and transfer of the sample to ^{2}D column [21]. Modulation also acts as a signal enhancer by zone compression [22]. The entire ^{1}D chromatogram is thus 'sliced' following a modulation period (P_M) of a few seconds and sent into ^{2}D for a fast GC-type separation [23]. By fine tuning of the GC phase combination, compounds potentially still co-eluting at the end of the ^{1}D can be separated on the basis of their different behaviors as regard of the ^{2}D phase. The separation power is increased and the sensitivity is enhanced [24]. For the detector responsible for recording the signal, everything happens as in classical GC and a trace is monitored continuously. In practice, series of high speed secondary chromatograms of a length equal to P_M (3–10 s) are recorded one after another. They consist of slices that can be combined to describe the elution pattern by means of contour plots in the chromatographic separation space [25]. A software is responsible for processing the collected raw data and extract the multi-dimensional information. A complete description of $GC \times GC$ instrumental setup is available in a previous report [26]. $GC \times GC$, often coupled to fast acquisition TOFMS, has thus been used to analyze complex samples in various fields, including VOC analyses [27–29].

To the best of our knowledge, despite the complexity of samples, $GC \times GC$–TOFMS has never been used in the geoforensic field. Here, we report preliminary results on using $GC \times GC$–TOFMS for the study of VOCs released in soil by buried decaying pig carcasses. The use of both retention times from the first dimension column, ^1t_R, and from the second dimension column, ^2t_R, peak intensity, deconvoluted mass spectral data as well as classification, scripting and simple chemometrics are combined to try to extract specific information about the composition and potential variations of the VOC mixture.

2. Experimental

2.1. Field site

The study site was a forest biotope, located in Belgium (Lambert-coordinates: 172800.00/167150.00). The field site was a research facility area devoted to forensic research managed by the Disaster Victim Identification (DVI) of the Belgian Federal Police. The tree layer of the field site was dominated by oak trees (*Quercus rubra*, *Quercus robur*) and beech trees (*Fagus sylvatica*). The shrub layer was absent. The soil vegetation was scattered and the herb layer was mainly constituted by bracken (*Pteridium aquilinum*), blackberry (*Rubus fructicosus*), lily-of-the-Valley (*Convallaria maialis*) and May Lily (*Maianthemum bifolium*) during spring and summer. Concerning the moss layer, there were some spare spots of *Polytrichum* sp. The Belgian soil map indicated a dry sandy loam with strong drainage. The soil profile consisted of a humus topsoil A-horizon (pH 2.1), which overlaid a subsoil B-horizon (pH 3.9), with clear eluviation and illuviation layers. The environmental parameters were monitored using two data recording methods, semi-local weather data and microclimate data. Semi-local weather data (atmospheric temperatures and precipitations) were obtained from the nearest weather station (at 6.5 km of the experimental site) of the Royal Meteorological Institute of Belgium (KMI-IRM). Microclimate data were recorded using a tinytag dual channel temperature logger (TGP1520®, Gemini Dataloggers, Chichester, UK). One probe was used to measure temperature variations in the core (anus) of the pig's body and the other probe was placed 5 cm beneath the ground surface of the grave fill.

2.2. Animal model

Two pig (*Sus domesticus* L.) carcasses were used to model the human decomposition process [30–33]. Both carcasses were obtained within 10 days of death. In February 2008, the pig carcasses were buried in shallow graves dug by hand, at a depth of 40 cm and on their left side (as part of another cadaver decomposition forensic study [34]). The pig graves were backfilled within 3.5 h with the excavated soil in random fashion and compacted by gentle trampling. The same was performed with a single control pit used for comparison with both carcasses. The spacing between the graves was just over 1 m. To avoid any vertebrate scavenging, a metal fence was placed around the grave site. After six months of burial, the graves were excavated by hand (at the beginning of August 2008) by a forensic investigation team (Fig. 1).

2.3. Sampling procedure and collection of VOCs

After six months of burial, gravesoil samples and control pit samples (300 g of soil) were collected at different depths during the pig excavation. Soil samples were taken at 5 cm, 10 cm, 20 cm, 30 cm (sample above carcass) and 40 cm (sample below carcass) from both the pig graves and control pit for blank collection. Soil samples were hermetically packed in zip lock plastic bags, transferred to the laboratory and conserved at −80 °C until collection of the volatile fraction was carried out in laboratory conditions (21 °C). The size of sampling bags was selected to minimize the headspace. A dynamic sampling was used to collect VOCs released by gravesoil samples placed inside a closed volatile collection device (Fig. 2). The device was made of a pump (MSA, Escort Elf pump) pulling air through the sampling device at a constant rate of 0.5 L/min for 1 or 2 h after 15 min of equilibration time. Gravesoil samples (50 g)