УДК 543.054

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Forensic cadaveric decomposition profiling by GC×GC-TOFMS analysis of VOCs

Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS) has been used to analyze complex mixtures of volatile organic compounds (VOCs) produced during cadaveric decomposition processes. The use of specific mass spectrometric scripting approaches permitted to easily identify gravesoils from control soils. The high peak capacity of the system, as well as the development of a specific data processing approach allowed the isolation and identification of several hundreds of specific analytes. When coupled to thermal desorption (TD), GC×GC-TOFMS is the tool of choice for cadaveric VOC profiling.
Key words: Forensics; VOCs; Cadaveric decomposition; Geotaphonomy; GC×GC-TOFMS; Profiling.

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ҰОҚ анықтаудың ГХхГХ-УҰМС көмегімен өліктің шіруінің криминалистикалық пішімдеуі

Өліктің шіруі кезінде түзілетін ұшқыш органикалық қосылыстардың күрделі қоспаларын талдауына уақытпен ұшып өтетін масс-спектрометрі бар екіөлшемді газды хроматография (ГХхГХ-УҰМС) қолданылды. Өліктің шіруінің әр түрлі сценарийлерін зерттеудің спецификалық масс-спектрометрлі тәсілдерін қолдану жерленген орынның топырағы мен салыстырмалы топырақтарды дифференцияциялауға мүмкіндік берді. Жүйенің жоғары шыңдық қуаттылығы және мәліметтерді өңдеудің белгілі тәсілдерін жасау спецификалық аналиттердің бірнеше жүздігін бөлуге және анықтауға мүмкіндік берді. Термодесорбциямен (ТД) үйлескен, ГХхГХ-УҰМС өліктің шіруінің ҰОҚ пішімдеуінің қолайлы құралы болып табылады.
Түйін сөздер: криминалистика, ҰОҚ, өліктің шіруі, геотаофономия, ГХхГХ-УҰМС, пішімдеу.

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Криминалистическое профилирование трупного разложения методом ГХхГХ-ВПМС определения ЛОС

Всеобъемлющая двухмерная газовая хроматография с времяпролетной масс-спектрометрией (ГХ×ГХ-ВПМС) была использована для анализа сложных смесей летучих органических соединений (ЛОС), образующихся при процессах трупного разложения. Использование специфичных масс-спектрометрических подходов изучения различных сценариев разложения позволило легко дифференцировать почвы с мест захоронения и контрольные почвы. Высокая пиковая мощность системы, а также разработка конкретного подхода обработки данных позволили разделить и идентифицировать нескольких сотен специфичных аналитов. В сочетании с термодесорбцией (ТД) ГХ×ГХ-ВПМС является предпочтительным инструментом для профилирования трупных ЛОС.
Ключевые слова: криминалистика, ЛОС, трупное разложение, геотаофономия, ГХ×ГХ-ВПМС, профилирование.

Introduction

Amongst the various fields of application of comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS), the analysis of biological samples for target or screening purposes represents quite a growing area of interest. Both signal and peak capacity enhancement properties of the technique are challenged through strategies ranging from, for example, low pg target analyses of selected persistent organic pollutants (POPs) in human matrices [1] to characterization of variations in complex metabolite fingerprint as a result of the progression of a disease [2].The analysis of volatile organic compounds (VOCs) released from various biological sources is also a challenging area of research, especially in the field of cadaveric decomposition...

Soon after death, the decay process of mammalian soft tissues begins and leads to the release of hundreds of cadaveric VOCs in the surrounding environment. The study of postmortem decomposition products is an emerging field of study in forensic science. However, a better knowledge of the smell of death and its volatile constituents is required for further applications in forensic sciences.The complex processes of decomposition produce a variety of chemicals as soft tissues and their component parts are broken down. These decomposition by-products include the VOCs responsible for the odor of decomposition. Human remains detection (HRD) canines utilize this odor signature to locate human remains during police investigations and recovery missions in the event of a mass disaster. Currently, it is unknown what compounds or combinations of compounds are recognized by the HRD canines. Furthermore a comprehensive decomposition VOC profile remains elusive. This is likely due to the difficulties associated with the non-target analysis of complex samples.

GC×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). GC×GC can be defined as a chromatographic technique during which a sample is subjected to two different separation processes coupled on-line [3]. The GC×GC principle is illustrated in Figure 1. In practice, the first dimension (^{1}D) column is serially connected to a second dimension (^{2}D) column of which the few first centimeters are placed in a temperature controlled interface named 'the modulator'. The cryogenic modulator ensures high sampling rates and transfer of the sample to ^{2}D column [4]. Modulation also acts as a signal enhancer by zone compression [5]. 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 [6]. By fine tuning of the GC phase combination, compounds potentially still co-eluting at the end of the ^{1}D separation can be separated on the basis of their different behavior as regards of the ^{2}D phase. Globally, the separation power is increased and the sensitivity is enhanced [7]. 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 seconds) 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 [8]. A software is responsible for processing the collected raw data and extract the multi-dimensional information. A complete description of GC×GC instrumental setup is available in a previous report [9]. Because of the very narrow peaks (<200ms), GC×GC is often coupled to fast acquisition TOFMS to ensure proper characterization of the ^{2}D peaks. GC×GC-TOFMS is thus a powerful tool to analyze complex samples in various fields, including VOC analyses [10,11,12].

For the last couple of years, we investigated the use of GC×GC-TOFMS for cadaveric decomposition studies. The approach has been based on sampling of the cadaveric headspace by means of trapping of VOCs on thermal desorption (TD) tubes packed with sorbents such as Tenax® and Carbopack™. The additional peak capacity of GC×GC, the spectral deconvolution algorithms applied to unskewed TOFMS signals, and the use of a robust data mining strategy allowed for the generation of a specific profile of decomposition VOCs across the various stages of soft-tissue decomposition. Several hundreds of postmortem compounds have thus been identified.

Experimental

Samples and field experiments. Because of their similarities to humans, domestic pigs have been used in most studies to surrogate human models [13]. Details on the field trail settings are available elsewhere [14,15]. The accumulated degree days (ADD) for each sampling day was calculated by summing the average daily temperature (°C). For

grave soil experiments, a forest biotope was selected [16]. Soils were sampled at various depths.

VOC collection. A dynamic sampling technique was used to collect volatile organic compounds released by the decaying pig carcass. The VOCs were collected in the headspace of the decaying pig or soil with a pump device for 0.5 to 1 hour at 0.2 to 1 L/min every day during the field experiment. Simultaneously to the cadaveric VOC collection, air samples were collected as blank references. Two approaches were used. First, VOCs were trapped on cartridges, constituted of glass and Teflon®, containing a 40 µg SuperQ® adsorbent filter (80-100 mesh, Alltech Associates, Inc. Deerfield, IL, USA). In the laboratory, VOCs were solvent eluted from the SuperQ® adsorbent with 150 µl of diethyl ether (HPLC grade, Sigma-Aldrich SA, Bornem, Belgium) and capped in GC-type vials. Before chromatographic analyses, liquid volatile samples were conserved at -80°C. A second approach consisted in covering the remains with a stainless steel hood measuring. The hood was fixed with a stainless steel bulk head connector which fastened the sampling tube to the hood creating a continuous path from the inside of the hood to sample the VOCs. During sampling, one end of the sampling tube was fixed to the bulk-head union and the other was connected to a LaMotte (Chestertown, Maryland, United States) model BD constant flow air sampling pump. Approximately 1 L of headspace was collected onto a multi-sorbent thermal desorption tube containing Tenax® GR (2,6-diphenylene-oxide and 30% graphite) and Carbopack™ B (poly-ethylene glycol and non-silicone phase coating) (Markes International Ltd, Llantrisant, UK) at a rate of 0.2 L/min. Following sample collection, tubes were capped with brass long-term storage caps fitted with PTFE ferrules and placed in a sealed mason jar for storage. Prior to desorption and analysis, 1 µL of a 60 ng/µLbromobenzene/methanol internal standard (IS) was added to the sampling tube using a standard GC injection syringe. Chemicals were purchased from Sigma-Aldrich® (Schnelldorf, Germany).

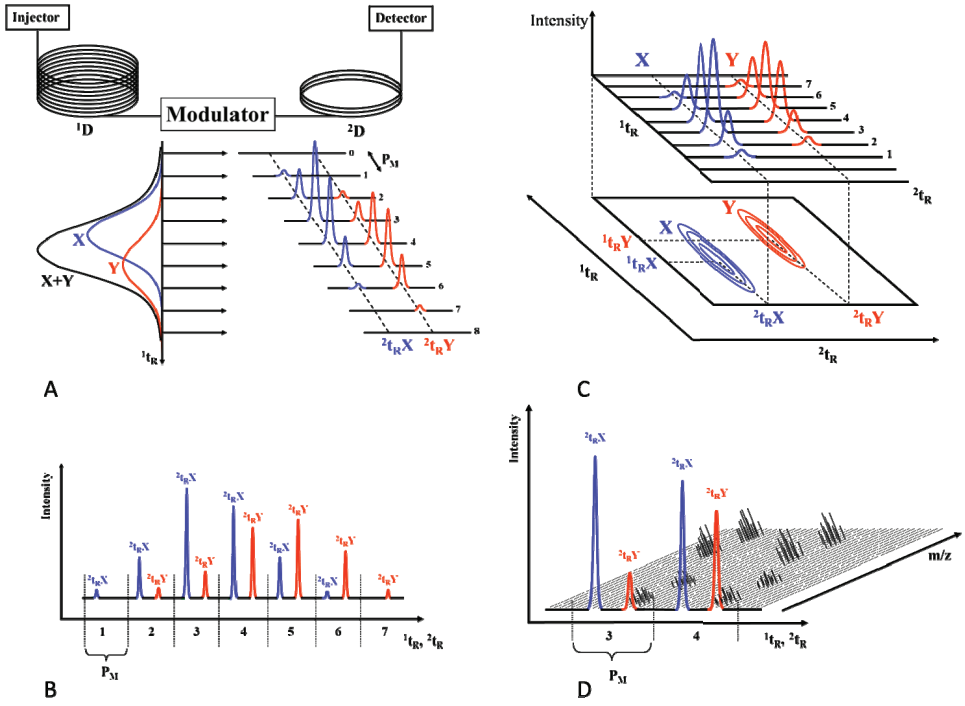

Figure 1 – Scheme of the column coupling in the GC×GC-TOFMS setup and how data are handled (not to scale). A: The modulator allows rapid sampling of the analytes eluting out of ^{1}D and reinjection in ^{2}D. The modulation process is illustrated for two overlapping compounds (X and Y) coming out of ^{1}D at a defined first-dimension retention time (^1t_R). As the modulation process occurs during a defined modulation period (P_M), narrow bands of sampled analytes are entering ^{2}D and appear to have different second-dimension retention times (^2t_RX and ^2t_RY). B: Raw data signal as recorded by the detector through the entire separation process. C: Construction of the two-dimensional contour plot from the collected high-speed secondary chromatograms of B), in which similar signal intensities are connected by contour lines. D: Expanded view of region 3 and 4 of C. The m/z axis lines illustrate the fast TOFMS acquisition process [20]

Measurements. The GC×GC-TOFMS instrument was the unit-mass resolution Pegasus 4D (LECO Corp., St Joseph, MI, USA). All details regarding GC and MS parameters are available in previous reports [14,15]. Briefly, thermal desorption and injection of samples was carried out using a Markes International Ltd. Unity 2 series thermal desorber. The sample tube was desorbed at a temperature of 300°C for 5min. The modulator was mounted in an Agilent 7890 GC gas chromatograph equipped with a secondary oven and a quad-jet dual stage modulator. Liquid nitrogen was used to create the cold jets. The P_M was 4 s with a hot pulse duration set at 700 ms and a cooling time between stages of 1300 ms. Carrier gas was helium and a constant flow of 0.8 mL/min was used. The GC column set used was made from the combination of a 30 m x 0.25 mm I.D. Rxi-5Sil, 5% phenyl 100% dimethylpolysiloxane (Restek Corp., Bellefonte, PA, USA) with a film thickness of 0.25 μm as ^{1}D and a more polar 1.2 m x 0.10 mm I.D. mid polar BPX-50, 50% Phenyl polysilphenylene-siloxane (SGE, Austin, TX) with a film thickness of 0.10 μm as ^{2}D. The transfer line connecting the secondary column and the MS source was operated at a temperature of 250°C. The source temperature was 250°C, operated in electron ionization mode with a filament bias voltage of -70 eV. The data acquisition rate was set at 100 spectra/s for a mass range from 29 to 450 m/z. The detector voltage was 1500 V. Daily mass calibration and tuning were performed using perfluorotributylamine (PFTBA).

Data Processing. GC×GC-TOFMS data were first acquired and processed with the ChromaTOF™ (4.42) software (LECO Corp.). This software was used for peak apex finding, mass spectral deconvolution, library searching, and integration. The combination of slices corresponding to a compound was performed by comparing the mass spectra under pre-established match criteria. Wiley (2008) and National Institute of Standards and Technology (NIST, 2008) databases were utilized for spectral identifications with a match factors threshold >700. A detailed description of the raw data processing procedure can be found in previous reports [14,17]. Following alignment of data processing tables, statistical information from various peak calculations was compared from each class and between classes. Fisher ratios were calculated from the compound table for each analyte in order to identify compounds showing the highest variance. The IS was used to calculate peak area ratio in order to normalize peak areas prior to statistical analysis [18]. Results were finally exported as .csv files and applied to external principal components analysis (PCA) software. PCAs were conducted using PAST 2.14 statistical software.

Results and discussion

Geoforensic

In case of suspicion of the presence of clandestine graves, upper layer soils can be used to highlight the presence of cadaveric VOCs. For this forensic geotaphonomy application (Figure 2), 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. The major challenge was to extract these marker molecules from the large amount of matrix related molecules. As illustrated in Figure 3, direct visual comparison of GC×GC-TOFMS chromatograms of a gravesoil to a control soil does not allow to spot significant differences. More than 150 peaks were present in each samples. Nevertheless, after data processing, some 20 specific compounds were specifically found in the soil sample taken under the carcass and 34 other compounds were found at various depths of the gravesoil samples. The group of the 20 compounds was made of ketones, nitriles, sulfurs, heterocyclic compounds, and benzene derivatives like aldehydes, alcohols, ketones, ethers and nitriles. The group of the 34 compounds was made of methyl-branched alkane isomers including methyl-, dimethyl-, trimethyl-, tetramethyl-, and heptamethyl- isomers ranging from C12 to C16. 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 differentiate soils at proximity of buried decaying pig carcasses from control soils (Figure 4) [16].

Figure 2 - Pig carcass after six months of burial at 80 cm depth [16]

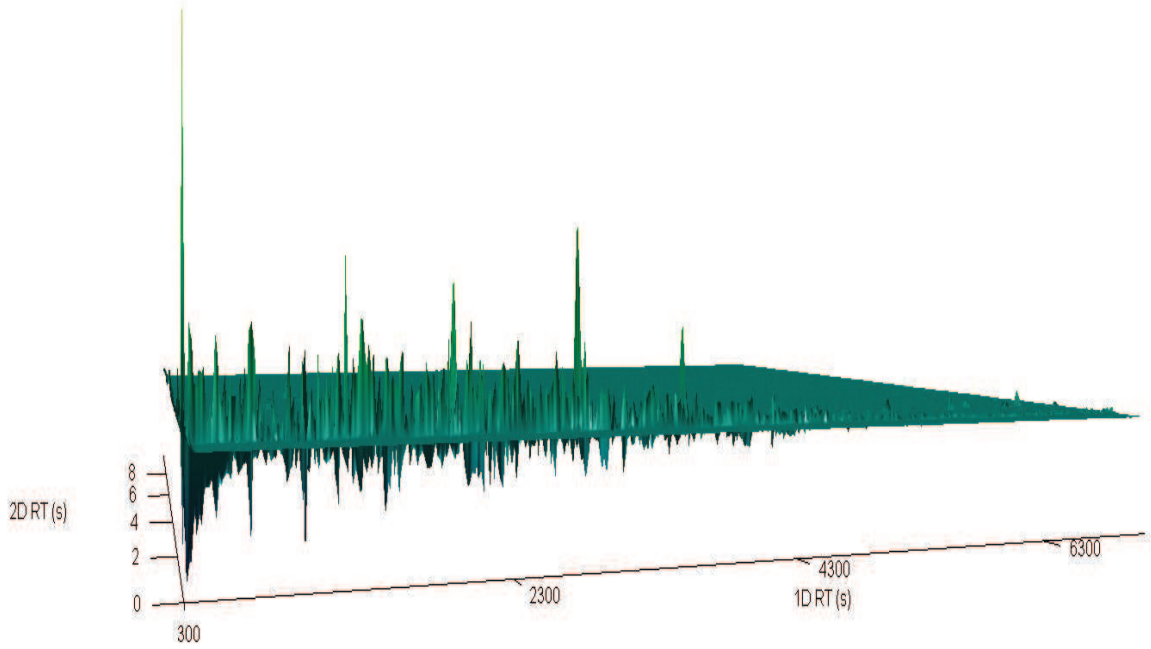

Figure 3 - GC×GC-TOFMS shape surface plot for a gravesoil sample (top) and a control soil sample (bottom)

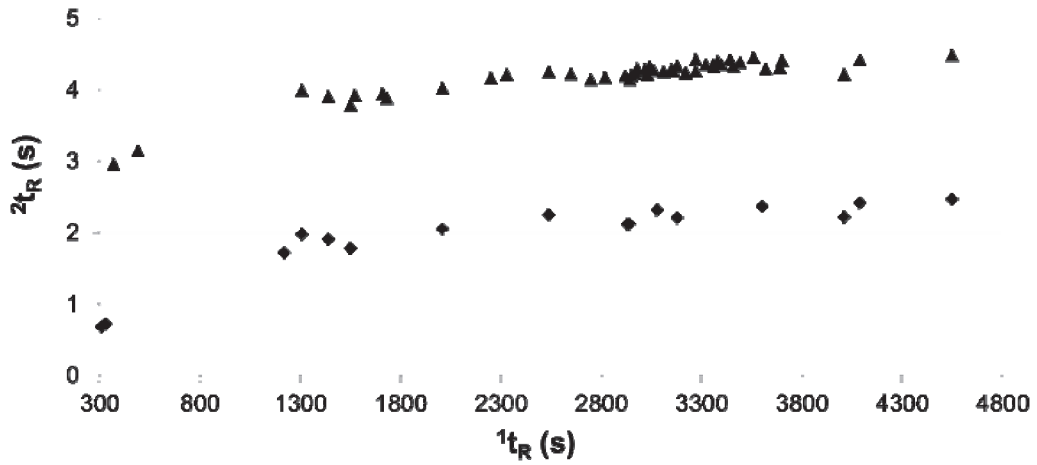

Figure 4 – Apex plot for both gravesoil and control results after scripting of soils at 10 cm depth (S/N of 200). Diamonds are for control soil (n = 16 hits), and triangles are for gravesoils (n = 47 hits). Gravesoil 2tR have been shifted of 2 sec for clarity [16]

Human analogs, Animal model

Complex processes of decomposition produce a variety of volatile chemicals as soft tissues and their component parts are broken down. Cadaveric VOCs produced by pig carcasses in decomposition were collected and thermally desorbed on GC×GC-TOFMS. The various stages of decomposition were followed and analyzed separately (Figure 5). TD-GC×GC-TOFMS complex data were specifically treated to extract the most appropriate information. Data reduction allowed to reduce the number of possible hits found from several thousands to less than 900 identified analytes. Figure 6 illustrates the procedure. During alignment, the data processing software utilizes algorithmic comparisons of peak features (^1t_R, ^2t_R, and mass spectra) to correct for variations during cross-sample comparison [19]. In order to identify meaningful compounds during non-target investigations, both chromatographic and spectral information within the data sets are required for subsequent statistical analysis.Following initial processing using ChromaTOF™ software, including spectral deconvolution and MS library searching, the statistical comparison feature of the software was used to compare the VOC profile of pig and control samples for each sampling day. It relies on a mass spectral match criterion of 60% to align multiple chromatograms and integrate peak area.Fisher ratios were calculated to highlight unknown chemical differences among known classes of samples. The aligned data sets were exported to Microsoft Excel spreadsheets for further data handling. Within Excel, relative peak area ratios were calculated against the IS and compounds were grouped into one of the 11 following chemical classes: alcohols, aldehydes, aromatics, carboxylic acids, esters, halogens, hydrocarbons, ketones, nitrogens, sulphides and others. In practice, the initial data processing identified approximately 10,000 hits over the entire set of experimental chromatograms. These hit tables included VOCs issued from both the environment and the pig decomposition, as well as instrument related signals such as column bleeds and multiple artifact hits resulting from various levels of peak tailing. Following data reduction and pre-statistical treatment, the data matrix contained above 300 peaks that were found to be specific to decomposition as present at least in one experimental day. This matrix was used to perform PCA. The number, types, and the distribution of compounds over post-mortem interval (PMI) are similar to those found by previous researchers [14].However, the use of the statistical comparison tool allows increased automation and is less time consuming. In particular, this tool utilizes raw peak tables generated by the initial ChromaTOF™processing, therefore eliminating manual screening and handling of the data.Figure 7 represents the relative abundance of the major chemical classes found during the decomposition process. A complete list of all analytes is available elsewhere [15].

Figure 5 – Typical decay stages followed by the pig carcass in a forest biotope [14]

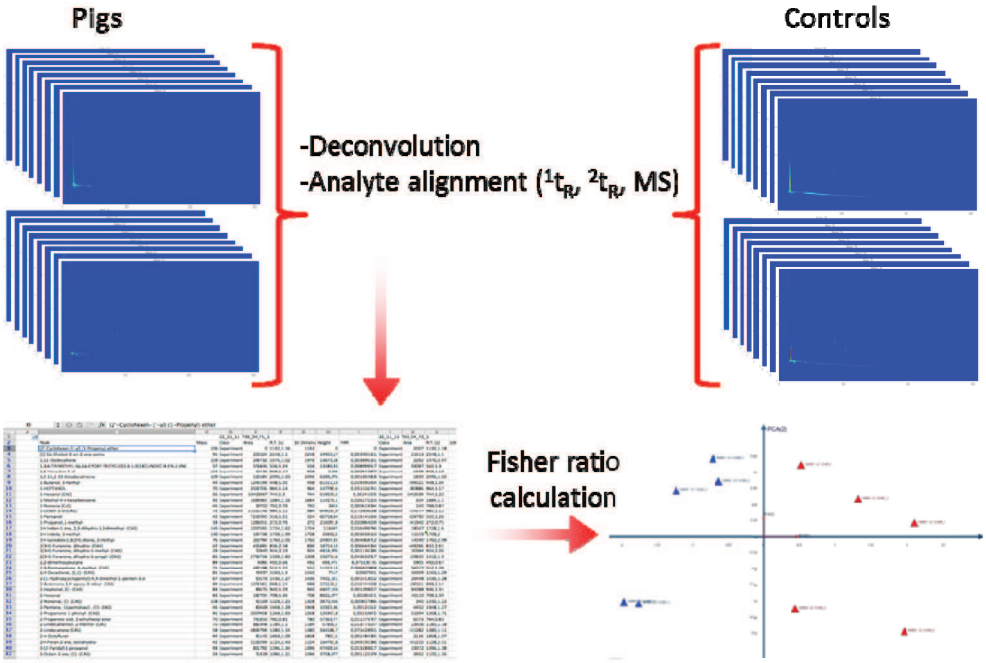

Figure 6 – Data treatment procedure using statistical alignment of all the chromatograms based the two retention times alignment and on mass spectra comparison, followed by PCA based on Fisher ratio selected above a certain threshold value [15]

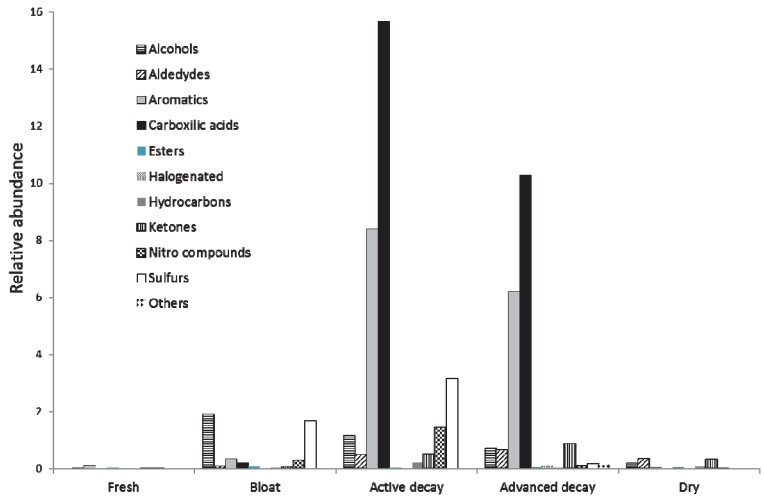

Figure 7 - Relative abundances of VOC compounds within the dominant chemical classes

Human model

The VOC profile of early stage decomposition of human bodies was also investigated in order to estimate the degree of similarity between the animal model and the human model. We analyzed samples collected during different trials organized during different seasons in a body farm located in Texas. Samples included environmental controls, pig carcasses, and human bodies (protected or not from scavenger insects). The data processing was performed in the light of identifying possible species variations. PCA treatment of the data highlighted some differences between species. Figure 8 shows that once the decomposition process started (Day 4 and Day 5), pig samples separate from human samples, both species being different from the control samples. In terms of analytes, current investigation of the sets of data shows that similar compounds are found for both species at various levels, but also that some compounds appear to be specific to either one or the other species. Further investigations on larger sets of samples are needed to further characterize these species.

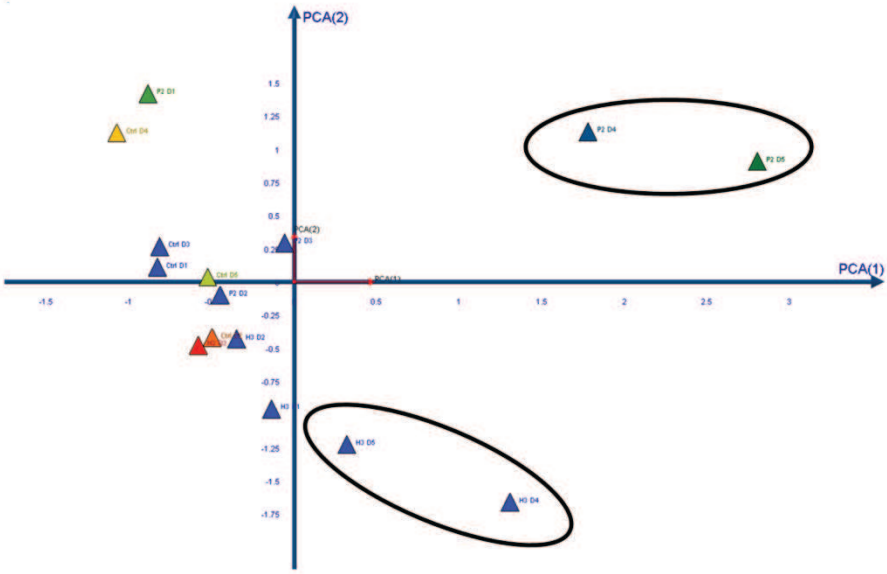

Figure 8 – Principal component analysis scatter plot of the PCA scores calculated for controls, pigs, and humans

Conclusion

TD-GC×GC-TOFMS is a powerful tool for the separation and identification of VOCs produced during cadaveric decomposition processes. The complexity of the VOC mixtures requires the implementation of state-of-the-art data processing strategies in order to extract practical information from the thousands of peaks. Several hundreds of molecules have been identified as specific from the cadaveric decomposition process. Specific scripting approaches appear to be useful for rapid sample screening. Further investigations are needed to gain more information in terms of comparison between the animal model and the human model. TD-GC×GC-TOFMS will surely significantly contribute to improve comparisons of complex VOC profiles in this specific area.

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