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On Target?

– for protein and peptide method development

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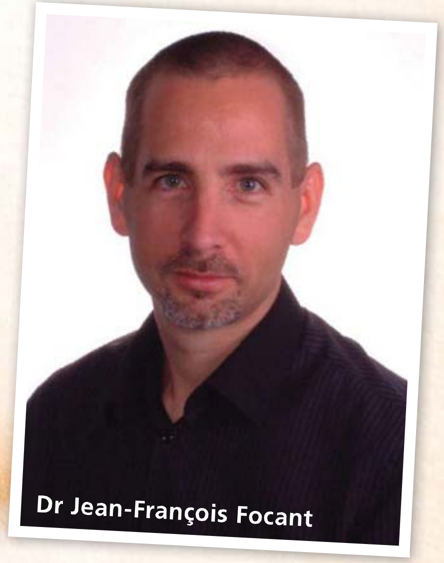
Developing the Sixth Sense

The Column spoke to Dr Jean-François Focant, associate professor at the chemistry department of the University of Liège, Belgium, about thenatochemistry and his recently published articles profiling the smell of death.

Q: What are your main research interests, and what led you to the field of thanatochemistry?

A: My primary research interests lie in the development of new chromatography strategies, coupling sample preparation procedures, introducing hyphenation to various types of mass spectrometric detectors through multidimensional systems and the implementation of emerging strategies under quality assurance/quality control requirements. The field of application for our groups' research encompasses human bio-monitoring, food control [both for persistent organic pollutant and complex volatile organic compound (VOC) mixture characterization]. Such VOC mixtures include, but are not limited to, combustion, chemical ecology, medical and forensic samples.

As a research group, working on the hyphenation of state-of-the-art analytical techniques to solve practical analytical issues is what we enjoy doing. Therefore, we became focused on the analytical challenge presented by the complexity of VOC mixtures associated with the 'smell of death' where we thought comprehensive two dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF–MS) methodology could be applied.[1]



Dr Jean-François Focant

Q: Can you outline how you develop your strategies?

A: At the beginning of our approach we used organic solvent to elute VOCs from traps; this was for the 'proof of concept'. Now we use a thermal desorber directly coupled to GC×GC (TD–GC×GC–TOF–MS) so that the VOC transfer is much more efficient and we remove the solvent peak issue.

The analytical strategy has been developed along two fronts. For both, initial efforts were dedicated to the optimization of GC×GC and TOF–MS parameters, including the development and optimization of an adequate data processing and handling procedure, to efficiently handle the large amount of peaks that were present in all samples. To ensure subtraction of background VOCs from specific analytes, statistical comparison tools were used.

For the first front, we analysed VOC mixtures sampled at close proximity to decaying pig carcasses, to increase the number of

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specific analytes identified and to perform better characterization of the various decomposition stages. Along the second front, we analysed soil samples taken at various depths close to graves. The idea was to identify marker analytes in surface soils that could be used in body-searching exercises. We also analysed some canine training aid solutions to complement those studies.

Q: What are the advantages of GC×GC–TOF–MS over other existing techniques? Are there any disadvantages?

A: The major advantages of GC×GC–TOF–MS are enhanced peak capacity and sensitivity, as a result of zone compression following modulation and deconvolution of MS signals. The coupling of GC×GC to TOF–MS therefore results in a powerful tool ideal for complex mixture analysis. This is especially true in cases where thermal desorption is used as a sample introduction device for VOCs. High chromatographic resolution and true peak deconvolution thus contribute to the separation of similar analytes that would not be separated using classical GC–MS systems.

Although cryogenic modulators are now very robust and routinely used, there is one major drawback. It is challenging to make sense of the complex data sets that are produced, although there are good software programs available now that help tremendously to process the large size data sets. The major challenge now is the post-processing statistical treatment using third party software. I find the more efficient the separation tool, the more complex the data.

Q: How have recent developments in chromatographic technology influenced the field of thanatochemistry?

A: The basic principle of GC×GC relies on the combination of GC phases that are as orthogonal as possible from one another, in order to work on different separation mechanisms; therefore, new GC phases are always welcome. This is especially true for more polar phases (generally not very thermally stable) that are typically used in second dimension (at relatively high temperatures). The introduction of ionic liquid phases has improved the range of phase combinations, as has the evolution of connectors (glass, metal, press tight connectors) all contributing to the development of reproducible methods in GC×GC-TOF–MS. The latest development in high resolution time-of-flight mass spectrometry (HR–TOF–MS) technology also opens the door to coupling GC×GC to HR-TOF-MS and accessing molecular formula information for proper peak identification.

In my opinion, I believe that the combination of thermal desorption sorbents in robust tubes is also of importance because they can enlarge the working volatility range.

Q: What are the challenges that you face when planning and performing studies profiling the 'smell of death'?

A: The major challenge is obtaining access to decaying bodies because of legal and ethical implications and that is one of the main reasons for using the pig model as a human surrogate. Locating an experiment field free of cadaveric VOCs is also challenging, as those fields are rare and have often already been used for similar decomposition studies. The trapping and analysis of VOCs is not challenging as our laboratory routinely runs GC×GC–MS methods.

Q: You say that pig corpses are commonly used as models because of ethical and legal considerations. How applicable is the data collected from pig carcasses to human corpses?

A: The answer depends on the person that you propose the question to. Most reports state that the pig model is the closest one to human, so studying VOC mixtures produced from pigs should be transposable to human studies. Some data indicates that this is the case; however, the field is still missing proper comparison between pig and human models. In my opinion, pig data most probably can help to understand the human decomposition process, but human data should be produced reproductively to confirm the similarity of the two models. As a matter of fact, our group are currently in the process of investigating VOC mixtures that were trapped in a body farm in the USA.

Q: In your recent work, you publish results showing a correlation between the composition of VOCs and the time post-mortem. Can you comment on the use of this data for forensics purposes?

A: The various stages of decomposition produce different sets of VOCs. Or more precisely, similar VOC profiles can be observed but the relative ratio between chemical families differs. So, if post-mortem interval (PMI) is calculated including temperature effect [average degree day (ADD) scale], one can imagine using this ratio variation to gain information on PMI by measuring VOC mixtures. The variation of predominant compounds classes' relative intensities definitely has potential application in forensic investigations.

Q: Could you comment on the application of your research for the potential development of a sensor?

A: Ultimately, our goal is for our data to be used for the development of sensors in the field. As it stands, our instrumentation is around 400 kg in mass and requires liquid nitrogen rangers to operate — not really something that you can carry in a backpack on-site. However, miniaturization is a possibility: cryo-free modulators and small size TOF–MS do exist.

A more realistic idea is to use GC×GC-TOF–MS to really narrow down the criteria of marker molecules to a very specific list that could then be transferred to portable 'e-nose' technology.

For criminal case investigations cadaveric research is obviously of interest, however, for me currently, extrapolating our research to living bodies is of even greater interest although the nature of VOCs collected are very different, as a dead body produces more VOCs than a living person. Nevertheless, on top of known fragrances, in response to danger the human body produces a selection of specific VOCs. Therefore, there is potential to use those specific VOCs to develop tracers to locate people trapped after, for example, a natural disaster. In that aspect, we probably still have a lot to learn from rescue and cadaveric dogs[2] and that is one of the reasons why we have investigated pseudo-solutions and will investigate that further in the future.

References

1. Jean-Francois Focant *et al.*, *Journal of Chromatography A*, **1255**, 163–170 (2012).
2. Jean-Francois Focant *et al.*, *PLos One*, **7** (6) (2012).

Jean-François Focant is currently an associate professor at the interfaculty Centre for Analysis of Trace Residue (CART) at the University of Liège, Belgium. He teaches chemistry to medical and veterinary schools, and is head of the organic and biological analytical chemistry group. Other scientific activities include temporary expert for the world health organization (WHO), invited expert for the European food safety authority (EFSA), cooperative teaching missions in Cuba, MS training and consultancy in the field of ultra-trace analyses.

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