GC×GC-HRTOFMS TO BETTER UNDERSTAND CLOAK-AND-DAGGER ACTIVITIES OF MADAGASCAR MANTELLA FROGS

P.-H. Stefanuto[a], C. Brasseur[a], C. Harisoa[b], M. Andriantsiferana[b], J.-F. Focant[a]

[a] Organic and Biological Analytical Chemistry Group, CART, University of Liège, Belgium
[b] Faculty of Science, Université Antananarivo, Madagascar

The Republic of Madagascar sits isolated 250 miles away from the east coast of Africa, in the Indian Ocean. Because of this isolation, several vegetal and animal species are endemic of the island, resulting in a unique reservoir of biodiversity. One typical representative amongst species of this typical wildlife is the Mantella frog genus [1].

Nowadays, there are 16 named species of Mantella frogs. However, some other species are waiting for the determination of their status. These frogs are currently endangered by the lost of their habitats and the poaching. These frogs are collected for the international pet trade for their small size (less than 5 cm) and the iridescent color of their skin. These aposematic colorations result from particular toxic secretions. Their skin poisons mainly contain mixtures of alkaloid molecules. These mixtures have been reported to vary according to geographical location, season, and diet of the frog [1-2].

The aim of this study was to develop an analytical procedure to characterize the alkaloids contents of the Mantella frogs' skin. Thirty samples were collected over a two-year period in different locations of the island. A liquid extraction process was developed to isolate the alkaloid mixture from the skin. Following this step, GC×GC-HRTOFMS was used to obtain a complete overview of the toxic mixture content. Finally, different statistical treatments were evaluated in order to highlight differences between skin samples.

This procedure allowed to obtain a clear overview of the frog skin secretion. As suspected, alkaloid mixtures appeared to be different from frog to frog depending of different parameters (species, time of collection…). The identified alkaloids will further be characterized and investigated to better understand cloak-and-dagger activities of Mantella frogs.

References

[1] F. Andreone et al., PLoS Biol. 6 (2008) e118.
[2] N. R. Andriamaharavo et al., J. Nat. Prod. 68 (2005) 1743.

PEAK DISPERSION EVALUATION AND OPTIMIZATION IN GC×GC

P.-H. Stefanuto[a], J.-M. Dimandja[b], J.-F. Focant[a]

[a] Organic and Biological Analytical Chemistry Group, CART, University of Liège, Belgium
[b] Department of Chemistry and Biochemistry, Spelman College, Atlanta, USA

From the early days of comprehensive two-dimensional gas chromatography (GC×GC), the concept of orthogonality has always been an important topic of discussion and debate. While the theoretical construct of orthogonality is fairly well understood and accepted, the practical evaluation of orthogonality in real GCxGC chromatograms has presented a bigger challenge in terms of finding consensus criteria amongst investigators. In fact, some recent studies have even suggested that orthogonality evaluation may not be as relevant as originally thought for GC×GC optimization. A more practical measure of bidimensional separation efficiency is the evaluation of the true peak capacity. The efficient use of the available peak capacity in a given separation is dependent on the stationary phase coupling, the operating conditions, and the relative dispersion of the peaks in the two-dimensional plane (1).

In this study, we investigated methods for peak dispersion evaluation and chromatographic quality criteria with the aim to contribute to the development of a statistical method for the optimization of GC×GC separation. The occupation of the two-dimensional space was evaluated using different approaches. First, geometrical methods were investigated: percentage of occupation (2), and convex hull ratio calculation (3). Second, the calculation of the dispersion was conducted using average Euclidian distance and standard deviation of this value. In order to evaluate, the quality of the chromatography, other criteria were also taken into account (e.g. the tailing factor).

In order to improve the robustness of this approach, we implemented a Box-Behnken surface response methodology. In this design of experiment, the peak dispersion and the chromatographic quality criteria were used as a response and the temperature ramp, the flow and the secondary oven offset were the variable parameters.

This approach demonstrated that peak dispersion and chromatographic criteria combined with a DOE provide a valuable way to optimize the GC×GC separation.

References

(1) S.K. Poole, C.F. Poole, The orthogonal character of stationary phases for gas chromatography, *J Sep Sci.* 2**008**, *31*, 1118–1123.
(2) Cordero, C.; Rubiolo, P.; Sgorbini, B.; Galli, M.; Bicchi, C. *J Chromatogr A*. **2006**, *1132*, 268–279.
(3) Semard, G. L.; Peulon-Agasse, V.; Bruchet, A.; Bouillon, J.-P.; Cardinaël, P. *J Chromatogr A*. **2010**, *1217*, 5449–5454.

CANADIAN CRAFT BEER CHARACTERIZATION USING TD-GC×GC-TOFMS

Pierre-Hugues Stefanuto[a*], Katelynn A. Perrault[a], Lena Dubois[a], Benjamin L'Homme[a], Catherine Allen[a], Caitriona Loughnane[a], Nobuo Ochiai[b], Jean-François Focant[a]

[a] CART, Organic and Biological Analytical Chemistry Group, Chemistry Department, University of Liège, Allée du 6 Août 11, B6c, Quartier Agora Sart-Tilman, B-4000 Liège, Belgium

[b] Gerstel K.K., 2-13-18 Nakame, Meguro-ku, Tokyo, 152-0031, Japan

For beer lovers, the worldwide tendency is to foster microbrewery style beer. The market is shifting from high volume pilsner beer to more unique varieties with higher alcohol content and a more complex character. Among small scale brewing, Belgian-style beer is encountering great success. In order to target similar specificity, international brewers can have access to yeast and hops commonly used by brewers in Belgium. However, even if the importance of these two factors is already established, they are unlikely to be the sole elements responsible for the popularity of the "Belgian touch".

In this study, thermal desorption coupled to comprehensive two-dimensional gas chromatography hyphenated with time-of-flight mass spectrometry (TD-GC×GC-TOFMS) was used to investigate the traditional Belgian beer style aroma profile and to compare it with a micro-scale Belgian-style beer production from Canada. Prior to the analysis, the chromatographic separation and the sampling parameters were statistically optimized using experimental design.

In order to obtain the most information from the complex GC×GC data matrix, advanced statistical tools were applied for comparing the different beer profiles. The major statistical approach was based on a Euclidian distance calculation to perform hierarchical cluster analysis (HCA). This allows multivariate investigation of the data with less stringent requirements in terms of the numbers of replicates, compared to other statistical methods.

This TD-GC×GC-TOFMS approach, combined with advanced statistical tools permitted the grouping of the different beers based on type and brewing method.