A METHODOLOGY OF SEMI-QUANTIFICATION IN COMPLEX MIXTURE USING DIRECT INFUSION FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

Introduction:

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) has proven to be an essential tool in the study of complex organic mixture, especially for petroleum or new energy products [1-3]. Due to these high performances in terms of resolution, mass accuracy, and dynamic range, this instrument allows distinguishing features according to their mass-to-charge ratio (m/z) and to assign a unique molecular formula to each signal (Figure 1) [4]. Thus, advanced and comprehensive molecular characterization at isobaric levels can be performed. This instrument is however not often used for quantitative studies, although the industrial demand is high.

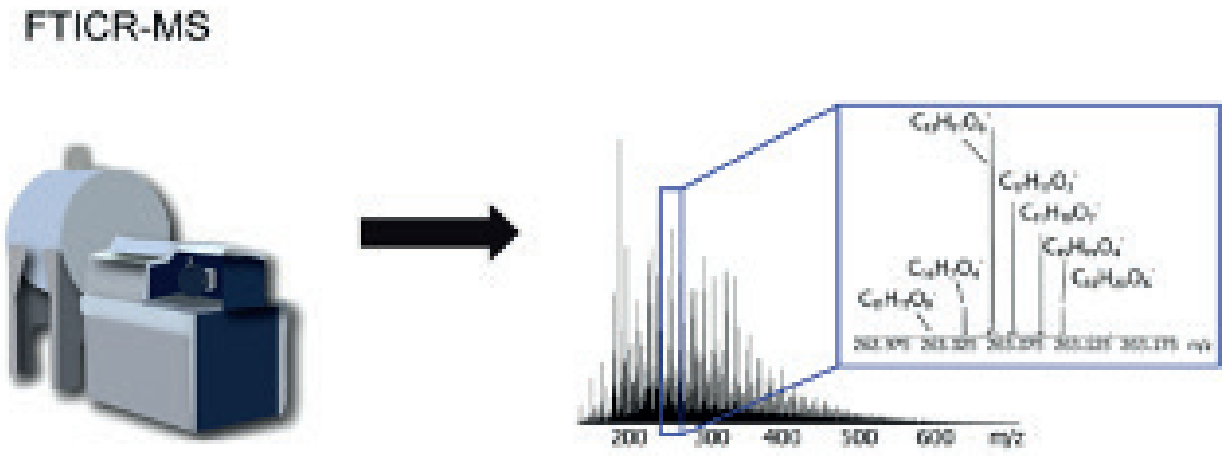

Figure 1. FTICR-MS as valuable tool for the molecular characterization of complex organic mixture

As the signals obtained in mass spectrometry are proportional to both the concentration and the response factor, calibration with standard molecules is necessary. This can be done by internal calibration [5], external calibration [6], and standard addition method [7]. For quantification, mass spectrometry is usually used with hyphenated separation methods such as liquid or gas chromatography. However, the huge amount of isomers can yield to a very low concentration for each individual molecule that could be not detected using chromatographic techniques. One way to overcome this issue is to perform direct infusion mass spectrometry as higher sensitivity is expected owing as isomers are not separated.

In this work, direct infusion ultra-high resolution mass spectrometry is used to perform a semi-quantification of molecules present in complex organic mixture. Standard addition method (SAM) was used in order to take into account matric effects.

Methodology:

General overview of the standard addition method:

The standard addition method is mainly used when the analyte to be quantified is in the presence of a complex environment leading to a strong matrix effect. The presence of other species can indeed lead to a significant modification of the signal of the analyte compared to the signal obtained with the analyte alone in solvent. Therefore, adding the standard directly to the sample overrides this eventual change in signal intensity. With SAM, the sample will be analyzed with known supplemental additions of the substance to be measured.

To be used, the standard addition method requires a linear relationship between the response factor and the concentration of the analyte with minimum of three points [8]. The concentration of the standard should be between 0.5 and 4 time the analyte concentration [9, 10]. SAM involves five main steps. The first step is the measurement of the analyte signal (Figure 2.1). Then, a known quantity of the standard was added (Figure 2.2). As the added volume is known, the concentration of the standard can be deduced. The intensity is measured after each addition (Figure 2.3) and reported as a function of the concentration on a graph ensuring that the additions remain within the linearity range (Figure 2.4). A too-high concentration range could lead to nonlinear response. The concentration of the analyte is finally obtained by extrapolation of the concentration absolute value obtained at the x-axis intercept (Figure 2.5).

Application to a complex organic mixture:

Due to the high number of signals present in a complex organic mixture, the SAM cannot be performed on all signals especially since it is impossible to find all standard molecules. The approximation made in the case of complex mixtures is that all molecules belonging to the same molecular class have the same response factor. For example, all N_1-containing species (i.e. $C_xH_yN_1$ species) would present the same signal evolution. However, to be used, this approximation requires that molecules in the same class have a similar structure. Therefore, it is necessary to carry out a structural study on several molecules present in the sample before the quantitative analysis. This structural characterization is important to identify appropriate standard molecules.

Tandem mass spectrometry (MS/MS) is a way to access such structural information in complex mixtures. Typically, ions of interest were isolated with the quadrupole and fragmented afterward through collisional activation. In the case of complex mass spectra, the low resolution for mass selection afforded by

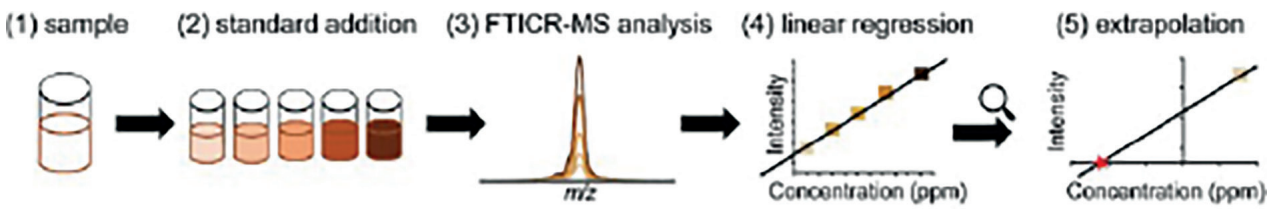

Figure 2. Methodology of the standard addition method

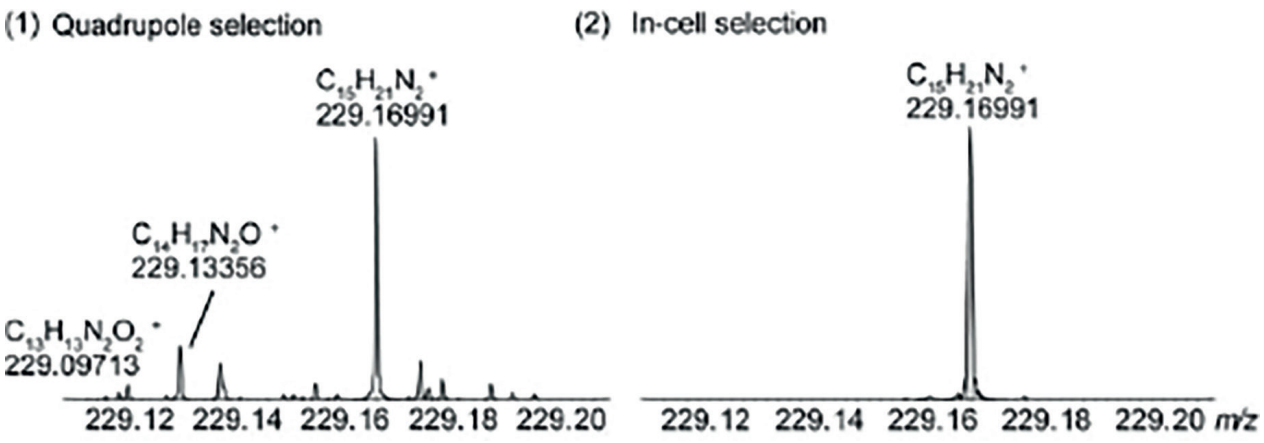

Figure 3. Comparison between isolations of m/z 229.16991 with (1) quadrupole selection and (2) in-cell selection

the quadrupole induces the co-isolation of many isobaric ions and the MS/MS spectra can be difficult to interpret (Figure 3.1) [11, 12]. However, FTICR mass spectrometers allow a high-resolution mass selection directly in the ICR cell using frequency-specific excitation pulses [13]. This allows a very accurate isolation of the ion of interest without the detection of isobaric ions (Figure 3.2).

Conclusion:

This approach was applied successfully for the quantification of nitrogen-containing species (N_1 and N_2 classes) in plastic pyrolysis oil from municipal waste. The structural characterization was obtained by tandem mass spectrometry using high-resolution mass isolation and infrared multiphoton dissociation fragmentation. Then, the semi-quantification was performed using 2-methylquinoxaline and 2-butylquinoline standard molecules [14]. The semi-quantification by direct infusion FTICR-MS for complex organic mixture was demonstrated for the first time. The reported methodology can be applied to numerous other families of compounds in various other complex mixtures.

Example of application to a plastic pyrolysis oil:

Mase, C., et al. (2022). "Speciation and semi-quantification of nitrogen containing-species in complex mixtures: application to plastic pyrolysis oil." ACS Omega.

https://doi.org/10.1021/acsomega.2c01114

References:

1. Marshall, A.G. and R.P. Rodgers, Petroleomics: chemistry of the underworld. Proc Natl Acad Sci U S A, 2008. 105(47): p. 18090-5.

2. Staš, M., et al., Petroleomic Characterization of Pyrolysis Bio-oils: A Review. Energy & Fuels, 2017. 31(10): p. 10283-10299.

3. Hertzog, J., et al., Characterization of Heavy Products from Lignocellulosic Biomass Pyrolysis by Chromatography and Fourier Transform Mass Spectrometry: A Review. Energy & Fuels, 2021.

4. Marshall, A.G., C.L. Hendrickson, and G.S. Jackson, Fourier transform ion cyclotron resonance mass spectrometry: a primer. Mass Spectrom Rev, 1998. 17(1).

5. Nilsson, L.B. and G. Eklund, Direct quantification in bioanalytical LC-MS/MS using internal calibration via analyte/stable isotope ratio. J Pharm Biomed Anal, 2007. 43(3): p. 1094-9.

6. Nunes, M.A., et al., External calibration strategy for trace element quantification in botanical samples by LA-ICP-MS using filter paper. Anal Chim Acta, 2016. 905: p. 51-7.

7. Garrido Frenich, A., et al., Compensation for matrix effects in gas chromatography-tandem mass spectrometry using a single point standard addition. J Chromatogr A, 2009. 1216(23): p. 4798-808.

8. Ceba, M.R., et al., Validation of an Analytical Instrumental Method by Standard Addition Methodology. Journal of AOAC INTERNATIONAL, 1995. 78(2): p. 471-476.

9. Saxberg, B.E.H. and B.R. Kowalski, Generalized standard addition method. Analytical Chemistry, 2002. 51(7): p. 1031-1038.

10. Ellison, S.L. and M. Thompson, Standard additions: myth and reality. Analyst, 2008. 133(8): p. 992-7.

11. Maillard, J., et al., Suggested plausible structures for Titan's haze analogs using tandem mass spectrometry. Icarus, 2021. 358.

12. Neumann, A., et al., Investigation of Island/Single-Core- and Archipelago/Multicore-Enriched Asphaltenes and Their Solubility Fractions by Thermal Analysis Coupled with High-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Energy & Fuels, 2020. 35(5): p. 3808-3824.

13. Vulpius, T. and R. Houriet, External phase shift ion ejection in fourier transform ion cyclotron resonance spectrometry. International Journal of Mass Spectrometry and Ion Processes, 1989. 88(2-3): p. 283-290.

14. Mase, C., et al., Speciation and Semiquantification of Nitrogen-Containing Species in Complex Mixtures: Application to Plastic Pyrolysis Oil. ACS Omega, 2022. 7(23): p. 19428-19436.

About the authors:

Charlotte Mase is currently conducting a PhD in analytical chemistry at COBRA laboratory in collaboration with TotalEnergies. She works on the molecular characterization of pyrolysis oils (plastic or biomass) and new sources of energies by ultra-high-resolution mass spectrometry

charlotte.mase1312@gmail.com

After receiving his PhD in analytical chemistry in 2000, at Paris VI University, **Carlos Afonso** joined the group of Prof. Catherine Fenselau, at Maryland University. In 2002, he obtained a CNRS researcher position in Paris. Since 2011, he is professor at Rouen University where he leads the mass spectrometry group of COBRA laboratory. His research interests are the development of new methodologies for the characterization of complex organic mixtures by FTICR MS and IM-MS.

carlos.afonso@univ-rouen.fr

Pierre Giusti obtained his PhD in analytical chemistry, from the University of Pau and Pays de l'Adour, in 2006. He is currently the manager of the Molecular Separation and Identification Service in R&D department of TotalEnergies Refining & Chemicals. Co-founder of the iC2MC laboratory, he also obtained the position of CNRS Research Director in January 2021. His research interests are the elemental and molecular analyses of complex matrices in the field of energy.

pierre.giusti@totalenergies.com

Author Contact Details

Carlos Afonso • University of Rouen, UMR 6014 COBRA / IRCOF, Rue Lucien Tesnière,

76130 Mont Saint Aignan, France • Tel: +33 2 35 52 29 40 • Email: carlos.afonso@univ-rouen.fr • **Web: www.lab-cobra.fr**

Charlotte Mase *Carlos Afonso* *Pierre Giusti*