DOI: 10.20396/revpibic.

Analysis of the metabolomic profile of volatile organic compounds in biological fluids by GC-MS.

Alessandra de Sousa Mesquita*, Ana Valéria Colnaghi Simionato.

Abstract

Metabolomics can be used for the analysis of volatile organic compounds (VOCs), which can be found in biological fluids and are synthesized in biological systems in normal metabolisms or in diseases, indicating their potentiality to be used as biomarkers. In this work, urine and blood serum samples from healthy individuals were used to optimize the sample preparation method by headspace solid phase microextraction (HS-SPME) followed by gas chromatography coupled to mass spectrometry (GC-MS).

Key words:

Metabolomics, Volatile Organic Compounds, Gas Chromatography Coupled to Mass Spectrometry.

Introduction

Lymphoma is a neoplasm that affects a group of cells of the immune system that have mutated. This neoplasm may be classified as Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL).[1] The patient's treatment plan is determined after some exams, such as: biopsy, anamnesis, physical exams, laboratory studies and imaging tests.[1] Patients can be treated with chemotherapy, monoclonal antibodies and radioimmunotherapy.[1] One possibility for diagnosis of lymphoid neoplasms is the monitoring of biomarkers synthesized and secreted into biological material (tissues or fluids).[2,3] Volatile organic compounds (VOCs) are produced by the organism and excreted in the human respiration, blood and urine, and may be used as biomarkers. VOCS extraction from biological samples may be performed by headspace solid phase microextraction (HS-SPME).[4,5]

Results and Discussion

GC-MS analyzes of urine and serum pooled samples from healthy subjects were carried out with a HP-5MS column (30 m × 0.25 mm × 0.25 µm), containing a 5% phenyl and 95% dimethylpolysiloxane stationary phase. Solid phase microextrations (SPME)[4,5] were performed with carboxene / divinylbenzene / polydimethylsiloxane fiber in the headspace (HS) of the vial containing the sample. The conditions of analysis were those indicated by the Fiehn library.[6] Extraction conditions optimization was performed by HS-SPME with a 2^3 factorial design. The number of molecular features (MF) was used to construct response surfaces.[7]

The 2^3 factorial design allowed evaluation of the interaction between three variables concomitantly at different levels, resulting in 17 assays – since the central point was analyzed in triplicate. Response surfaces were constructed for graphical interpretation in order to find the best experimental conditions – maximum number of MF.[7]

Urine samples: In factorial planning 1 the following variables were evaluated: x_1 - NaCl mass; x_2 - Extraction temperature; x_3 - Time of fiber exposure to HS. The best conditions were achieved with: x_1 = 0.5 g; x_2 = 90 °C; x_3 = 15 min (MF = 72). In factorial design 2 the evaluated variables were: x_1 - Equilibrium time; x_2 - Shaking time; x_3 - Sample volume. The maximum system response was obtained with the conditions: x_1 = 15 min, x_2 = 19 s, and x_3 = 3.5 mL (MF = 96).

Blood serum samples: Factorial designs 1 and 2 were evaluated accordingly. The optimum conditions obtained in 1 were x_1 = 1.5 g; x_2 = 90 °C; x_3 = 15 min (MF = 56); in 2, the best system response was obtained with x_1 = 24 min; x_2 = 23 s; x_3 = 0.5 mL (MF = 64). The chromatogram with the optimized conditions for the blood serum samples is presented in Figure 1. Some of the identified metabolites were verified in the *Human Matabolome Database*[8] platform, namely: 1-Dodecanol (11,31 min), Hexadecane (14,97 min) and Pentadecane (14.47 min).

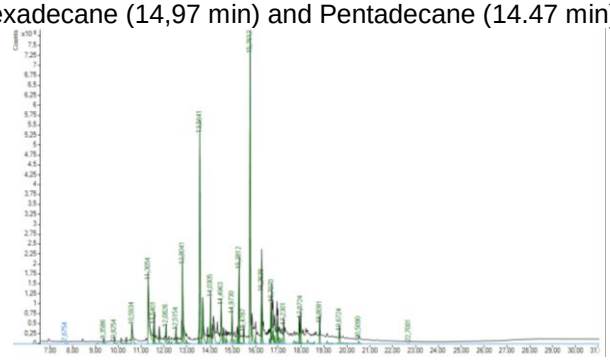

Image 1. Chromatogram obtained under optimized extraction conditions for blood serum samples analysis.

Conclusions

The response surfaces allowed obtaining of optimal experimental conditions for analyses of VOCs in urine and blood serum, evaluating only 3 levels of each investigated variable. Therefore, the interaction among the variables was considered with a reduced number of experiments.

Acknowledgement

Campinas' Hemocentro, FAPESP, CAPES and CNPq.

[1] Fisher, R. I.; Mauch, P. M.; Harris, N. L.; Friedberg, J. W. *Philadelphia: Lippincott Williams & Wilkins*, **2005**, 7th ed, 1957-7.
[2] Li, X.; Asmitanda, T.; Gao, L.; Gai, D.; Song, Z.; Zhang, Y.; Ren, H.; Yang, T.; Chen, T.; Chen, M. *Neoplasma*, **2012**, 59, 5.
[3] Kelly, R. S.; Vineis, P. *British Medical Bulletin*, **2014**, 111:89–100.
[4] Schmidt, K.; Podmore, I. *Journal of Molecular Biomarkers & Diagnosis*, **2015**, 6, 1-16.
[5] Augusto, F.; Valente, Microextração por Fase Sólida. *Química Nova*, **2000**,23, 523-530.
[6] Fiehn, O.; Kopka, J.; Trethewey, R. N.; Willmitzer, L. *Anal. Chem*, **2000**, 72 (15), 3573–3580.
[7] Breitkeitz, M. C.; de Souza, A. M.; Poppi, R. J. *Quim. Nova*, **2014**, 37, 3, 564-573, 2014.
[8] Wishart DS, Tzur D, Knox C, *et al*. HMDB: the Human Metabolome Database. Nucleic Acids Res. **2007**.