doi:10.20396/revpibic262018454

XXVI Congresso de Iniciação Científica Unicamp
17 a 19 de outubro Campinas | Brasil

Exploring methodologies to obtain the metabolomic profile of biological fluids by LC-MS/MS

Flávia P. Filliettaz*, Gustavo H. B. Duarte, Marcos N. Eberlin, Ronei J. Poppi, Ana Valéria C. Simionato

Abstract
The investigation of methodologies to obtain the metabolomic profile of blood serum from healthy individuals by LC-MS/MS was performed by factorial planning and the number of molecular features as the system answer. Sample preparation, chromatographic and ionization source conditions were optimized, defining the best conditions to obtain the metabolomic profile of biofluids to be applied in future identification of potential tumor biomarkers.

Key words:
Metabolomic profile, factorial planning, biological fluids.

Introduction

Metabolomics, the science that studies the metabolites in a biological system, may provide identification of possible biomarkers, which help to determine diagnosis, prognosis and prediction of diseases. The investigation of the metabolic profile, which is the total set of metabolites present in a biological sample, and its comparison between two groups of distinct samples (*e.g.*healthy and diseased), allows to highlight the metabolites that distinguish them, as well as the ientification of biomarkers of human diseases. For this purpose, the investigation of methodologies that best describe these profiles in biological fluids was performed in this work, where samples preparation, chromatographic and ion source conditions (electrospay - ESI) were optimized using factorial plannings. The number of molecular features (MF - potential metabolites) of each analysis was used to evaluate the best analysis conditions, which will be employed for later use in the identification of potential tumor biomarkers.

Results and Discussion

Untargeted metabolomics analyses of blood serum was optimized using a pool of samples from healthy individuals. Sample preparation, chromatographic and ion source conditions (ESI (+) and (-)) were studied. The results were evaluated as the number of MF, always seeking for the condition that maximize it, since it promotes the largest number of identified metabolites.

The preparation of serum samples was optimized focusing only on the precipitation of proteins. Therefore, the nature of the organic solvent (acetonitrile, methanol and isopropanol), solvent temperature (room temperature and cold), and the sample:solvent volume ratio (1:3 and 1:4 – v:v) were evaluated. A split plot experimental design was applied for this purpose. Chart 1 exemplifies some tests performed with blood samples and the best conditions.

Regarding reversed phase liquid chromatography conditions, evaluations with isocratic and gradient elution modes were performed. Image 1 shows the chromatogram where the highest number of molecular features were detected.

To optimize the conditions of the ionization source, the following variables were considered: capillary voltage, nozzle voltage, gas temperature, drying gas flow, sheath gas temperature, sheath gas flow, and nebulizer gas temperature, which were evaluated in 3 levels (maximum, minimum and medium) and the experimental design was a fractional factorial design (2^{7-3}). The results were evaluated as the number of MF as well.

Chart 1. Conditions for preparation of blood serum samples.

assay	Solvent	Temperature	Sample: Solvent	MF (ESI+)	MF (ESI-)
1	ACN	Room	01:03	76	92
2	ACN	Ice	01:03	73	90
3	ACN	Room	01:04	64	79
4	ACN	Ice	01:04	75	75
5	**MeOH**	**Ice**	**01:03**	**100**	**121**
6	**MeOH:ACN:Isop**	**Ice**	**01:03**	**90**	**146**

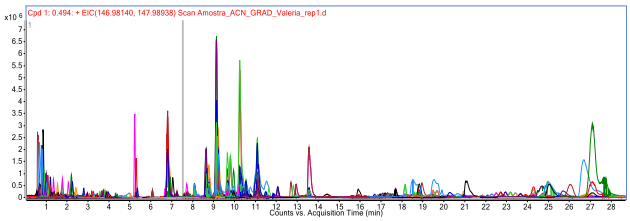

Image 1. Optimized chromatographic conditions for metabolomic analyses of blood serum samples.

Conclusions

According to the number of molecular features obtained by the analyzes and with the effects of the variables determined through the factorial planning, it was possible to obtain the best conditions for sample preparation (cold methanol for ESI(+) and cold MeOH:ACN:Isop for ESI(-) with a sample volume ratio of 1:3), chromatographic conditions (gradient elution) and ionization source conditions (ESI) (shown in detail on poster) for biological fluids. Such conditions will be used in future studies of tumor biomarkers.

Acknowledgement

To INCTBio, CNPq, FAPESP and Hemocentro Campinas

[1]Oliver, S.G.,*TrendsBiotechnol.*, 16, 373–378, 1998.
[2]Atkinson, A. J., Colburn, W. A., DeGruttola, V. G., DeMets, D. L., Downing, G. J., Hoth, D. F., Clinical Pharmacology and Therapeutics, 69(3), 89–95, 2001.
[3]Issaq, H. J.; Van, Q. N.; Waybright, T. J.; Muschik, G. M.; Veenstra, T. D., *J. Sep. Sci.*, 32, 13, 2183–2199, 2009.
[4]Tautenhahn, R., Patti, G. J., Rinehart, D., Siuzdak, G., *Analytical Chemistry*, 84, 5035-5039, 2012.