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Optimization of serum sample preparation aiming at metabolomics analysis by comprehensive two-dimensional gas chromatography coupled to mass spectrometry.

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Abstract

This work has the purpose of optimizing the analysis of serum samples by comprehensive two-dimensional gas chromatography coupled to mass spectrometry, using derivatization for sample preparation, and aiming at further application in metabolomics studies of bipolar disorder.

Key words:
Derivatization, gas chromatography, mass spectrometry, metabolomics.

Introduction

Metabolomics consists in the identification of small metabolites from complex biological matrices [1]. In this this work, a sample preparation method was optimized for analyzing serum aiming the future application in the study of bipolar disorder.

Comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-MS) is one of the most powerful techniques for analyzing the metabolome of complex mixtures because of its high resolution and high sensitivity. However, the low volatility of many metabolites limits the analysis to low molecular mass compounds [2]. This explains the importance of derivatization, since it provides thermally stable derivatives of high molecular mass compounds that are suitable for GC×GC-MS analysis [3].

Results and Discussion

Initially, a literature search about derivatization methods for metabolomics analysis of serum samples was performed. A two-step method using methoxyamine and a trialkylsilyl reagent was indicated to be the most suitable procedure.

Silylating reagents react only with polar functional groups that have a replaceable hydrogen atom [3], which is the reason for a two-step derivatization. In the first reaction, methoxyamine was used to protect the carbonyl functional groups by forming methoxyoximes [4].

In the second step, the derivatization reagent N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was used to increase the volatility of the compounds, as described in the work of Palazoglu *et al.* [1].

Proteins may interfere in the derivatization reaction; therefore, the sample preparation starts by precipitating the proteins [1]. This process was not enough to remove all the proteins from the samples, then Agilent Captiva EMR Lipid cartridges were employed as an additional sample preparation step.

The results were evaluated by the quantity of compounds found in the chromatogram. Figure 1 shows a representative chromatogram of a GC×GC-MS serum analysis.

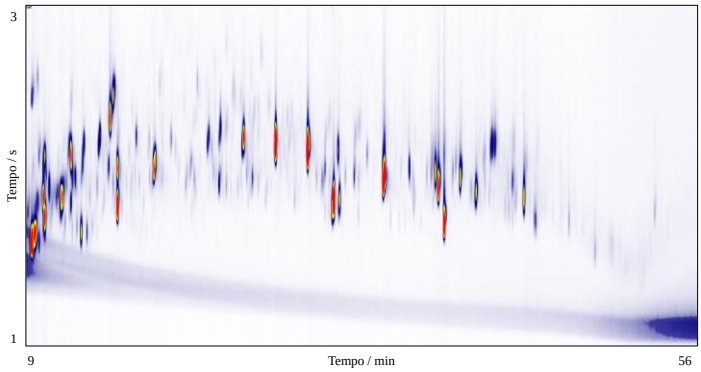

Figure 1. Two-dimensional GC×GC chromatogram of the derivatized serum samples.

Before derivatization, serum samples underwent a liquid-liquid extraction procedure, using water-chloroform-methanol. Thus, organic and aqueous phases were tested, where the first presented more compounds in the chromatogram and was considered the best phase to work with in subsequent analyses.

Conclusions

The main objective of this work was to optimize a sample preparation method to analyze human serum samples by comprehensive gas chromatography coupled to mass spectrometry. It was found that the Agilent Captiva EMR Lipid cartridge followed by a two-step derivatization was the method that provides the best serum metabolome overview, using the organic phase extract.

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