Multivariate Optimization of HF-MMLLE Sample Preparation for Simultaneous Extraction of N-Nitrosamines in Beverages

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INTRODUCTION

Nitrosamines or N-nitrosamines (NAs) are organic compounds consisting of a nitroso functional group (–N=O) bonded to a secondary amine, with a general chemical structure of R_2N–NO. Their presence in beverages may pose a risk to human health, as these compounds are potentially carcinogenic and mutagenic. Due to the trace levels of N-nitrosamines frequently found in these samples and their complexity, matrix interference isolation and pre-concentration of these compounds are essential steps before analysis.[1] In this study, the hollow fiber microporous membrane liquid-liquid extraction (HF-MMLLE) technique was optimized and used to pre-concentrate six N-nitrosamines.

RESULTS AND DISCUSSION

Identifying optimal experimental conditions for the simultaneous extraction of NAs is a complex task. Design of Experiments (DoE) is essential to obtain the best conditions that satisfy all analytes. In this study, a mixture design with a process variable was used to optimize solvent mixing and desorption time, using the chromatographic peak areas of NAs (N-nitrosopyrrolidine (NPYR), N-nitrosomethylethylamine (NMEA), N-nitrosopiperidine (NPIP), N-nitrosodiethylamine (NDEA), N-nitrosodi-n-propylamine (NDPA), and N-nitrosodi-n-butylamine (NDBA)) as the response. The Derringer & Suich desirability function was used to simultaneously optimize conditions using the GAMMA-GUI app.[2] Figure 1 shows that the optimized formulation achieved a D value of 0.75, in which the solvent proportions were adjusted to complete a volume of 300 µL consisting of 45.2 µL of MeOH, 110.5 µL of H_2O, and 144.3 µL of EtOH with an optimal desorption time of 20.4 min. After optimization of the desorption solvent and desorption time, a Box-Behnken design was used to optimize the extraction process of the analytes. In this step, the influence of pH (1, 4, and 7), NaCl concentration (0, 5, and 10%), and time (10, 20, and 30 min) on the microextraction process was investigated. The multi-response optimization for the extraction step was performed considering the four analytes, since the optimized results for three and four analytes were very similar (NDEA analyte shows a lack of fit). Figure 2 shows the response surfaces for the three analytes (NPYR, NMEA, and NPIP). The optimized formulation presented a D value of 0.80 under ideal conditions of pH 3.9, NaCl of 8.5%, and extraction time of 31.5 minutes. This condition was experimentally validated.

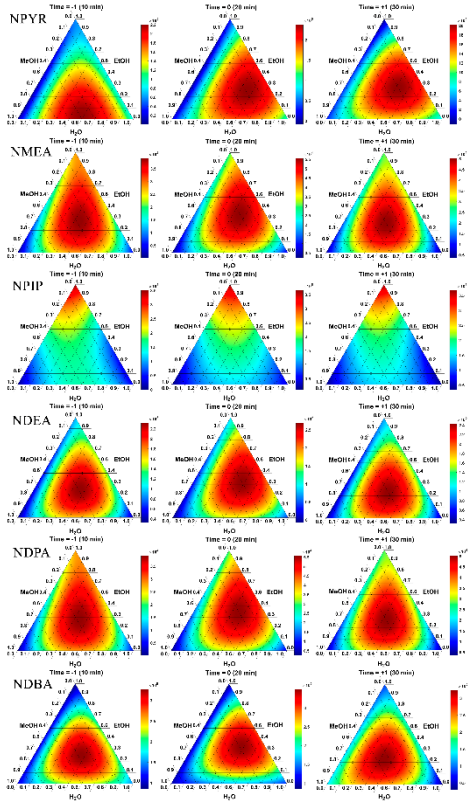

Figure 1. Response surface for the mixture design with a process variable.

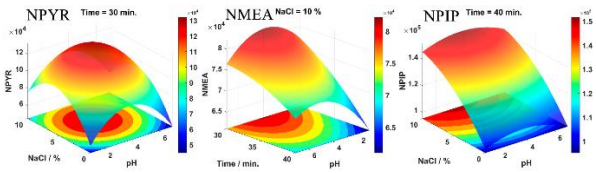

Figure 2. Response surface for the Box-Behnken design.

CONCLUSIONS

DoE was crucial for optimizing the HF-MMLLE sample preparation, determining the ideal solvent proportions and desorption time for NAs with a mixture design with a process variable. While Box-Behnken design was used to optimize the analyte extraction step.

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