DLLME: An Innovative Approach for Rapid and Low Cost Determination of Psychoactive Substances and Antibiotics in Wastewater

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Abstract

Wastewater-based epidemiology (WBE) has been demonstrated to be a valuable tool in the assessment of the health and well-being of a community, with the detection of various indicators by analysing the influent wastewater coming in Wastewater Treatment Plants. The accurate identification and quantification of analytes and their metabolites associated with daily life facilitate the extraction of conclusions regarding trends and the prediction of future consumption.

The analysis of wastewater necessitates a methodology that is capable of cleaning the sample and preconcentrating the analytes, which are present in low concentrations. A highly promising pretreatment method is DLLME due to its combination of rapidity and reduced cost. The objective of this study is to reveal the capabilities of DLLME in Wastewater Epidemiology.

An optimized DLLME method was developed for the monitoring of two significant classes of compounds: namely, psychoactive substances and antibiotics. This method was developed through the selection of the most suitable conditions and was subsequently validated. The validated method was applied to real samples from two different periods of time, and the results were statistically compared with the corresponding ones from the validated SPE method.

Keywords: DLLME, Wastewater- Based Epidemiology (WBE), Antibiotics, Psychoactive substances

1.   Introduction

The necessity to detect antibiotics and psychoactive compounds, even in trace amounts, in sewage necessitates the development of highly sensitive methods. The method for sample preparation and analysis combines the convenience and low cost of DLLME (Dispersive Liquid-Liquid Micro Extraction) with the ability to achieve low detection limits through the liquid chromatography-coupled mass spectrometry (LC-MS/MS) technique. This contributes to the ability to quantify analytes even at very low levels with simultaneous positive and negative ionization of the compounds.

During the study, several difficulties are encountered, such as (i) signal suppression due to matrix effect, as the wastewater is a complex matrix which includes a multitude of chemical compounds, (ii) the different nature of antibiotics and psychoactive substances related to their extractability, (iii) the different concentration levels of analytes in wastewater from different regions.

In the course of developing the method, the parameters that are critical to the application of DLLME were selected. The most suitable extraction and dispersion solvent and their ratio, the pH adjustment of the sample, its volume, the time of placement in ultrasonication and the selection of the amount of NaCl were the parameters that they were optimized.

Following the development of the method, its validation was undertaken by assessing linearity, trueness, repeatability, intermediate precision, detectability and uncertainty of the method. The validation experiments were performed on three separate days with pooled samples of influent wastewater from WWTPs of different Greek cities.

The validated method was applied for the analysis of real samples and was statistically compared to the validated method of the Laboratory of Analytical Chemistry based on SPE pretreatment.

2.   DLLME optimization

The critical parameters that exert an influence on the DLLME procedure were selected subsequent to the conduction of experiments and the calculation of absolute recovery. As was evidenced in the initial experiments, the most efficacious option was a two-step extraction process, in contrast to the one-step extraction method that had previously been employed. Prior to the DLLME procedure, influent wastewater samples were filtered using glass fiber filters with a 0.45 mm pore size. All samples were subsequently spiked with 5 uL of the internal standard working solution and the pH was adjusted to 9 with NH4OH 2% v/v. 50 mg of sodium chloride was added to achieve a concentration of 1% v/v in the sample. 2 mL of a mixture consisting of acetonitrile (dispersive solvent) and chloroform (extraction solvent) was rapidly injected into the sample creating a cloudy solution. Then it was

placed in an ultrasound bath for 5 min and then centrifuged at 4000 rpm for 10 min. The sedimented phase was collected by the bottom of the centrifuge tube and transferred to a test tube using a syringe of 1 mL. The pH of the aqueous phase remained in the centrifuge tube was adjusted to 2 with HCl 1 M. The steps from the pH adjustment to the collection of organic phase were repeated and the sedimented phase of the second extraction was transferred to the same test tube. The content of the test tube was evaporated with nitrogen stream, the residue was reconstituted with 100 uL acetonitrile/ water (1:1, v/v) 0.1% formic acid and the reconstituted samples were filtered by RC filters with a pore size of 0.45 um.

3. Validation of the method

The validation of the method was conducted subsequent to its optimisation, which involved the evaluation of various quality performance characteristics. Linearity ($R^2 \geq 0.98$), trueness (R = 80-120%), repeatability and intermediate precision (RSD $\leq$ 20%), detectability (LoD and LoQ of the class of ppt concentration) and uncertainty were satisfactory.

4. Comparison with SPE procedure

The method was applied to real samples in order to test its effectiveness. A comparison was made between the results and those of the SPE procedure, with the use of a paired t-test. The P-value for most analytes was found to be equal to or greater than 0.05. This indicates that there was no statistically significant difference between the results obtained from the two different methods. MDMA (P-value=0.011), Clindamycin (P-value=0.028) και Metronidazole (P-value=0.021) indicated P-values lower than 0.05 because most of the results were close to LoQ of the method.

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