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Use of experimental design in the investigation of stir bar sorptive extraction followed by ultra-high-performance liquid chromatography–tandem mass spectrometry for the analysis of explosives in water samples

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ABSTRACT

A method for the sensitive quantification of trace amounts of organic explosives in water samples was developed by using stir bar sorptive extraction (SBSE) followed by liquid desorption and ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS). The proposed method was developed and optimized using a statistical design of experiment approach. Use of experimental designs allowed a complete study of 10 factors and 8 analytes including nitro-aromatics, amino-nitro-aromatics and nitric esters. The liquid desorption study was performed using a full factorial experimental design followed by a kinetic study. Four different variables were tested here: the liquid desorption mode (stirring or sonication), the chemical nature of the stir bar (PDMS or PDMS-PEG), the composition of the liquid desorption phase and finally, the volume of solvent used for the liquid desorption. On the other hand, the SBSE extraction study was performed using a Doehlert design. SBSE extraction conditions such as extraction time profiles, sample volume, modifier addition, and acetic acid addition were examined. After optimization of the experimental parameters, sensitivity was improved by a factor 5–30, depending on the compound studied, due to the enrichment factors reached using the SBSE method. Limits of detection were in the ng/L level for all analytes studied. Reproducibility of the extraction with different stir bars was close to the reproducibility of the analytical method (RSD between 4 and 16%). Extractions in various water sample matrices (spring, mineral and underground water) have shown similar enrichment compared to ultrapure water, revealing very low matrix effects.

1. Introduction

Nitrated energetic compounds such as nitroesters, nitroaromatics and nitroamines are industrially produced in large quantities for mining, quarrying and civil engineering applications. Since most of these organic explosives and their degradation products are considered toxic [1], their release in the environment and the subsequent contamination of soil, groundwater and surface water, poses significant environmental and public health risks [2]. These organic explosives are also the major components of various types of arms, mines and ammunition worldwide. Manufacturing, training, open burning or detonation as well as improper disposal in landfills and sea dumps have led to the contamination of both terrestrial and marine environments [3]. For example, up to 2000 sites have been identified in the United States as potentially contaminated by explosives [4] and, in Canada about 100 open burning, detonation and/or training sites handle RDX, HMX and TNT [5]. In Germany, United Kingdom and Australia, munition manufacturing sites that were demolished immediately after World War II are being characterized and remediated [6]. As a result of these various civil, military, or even terrorist applications, reliable assessment of the presence of energetic compounds in the environment has become key issue for both forensics and environmental applications and sensitive methods are required in these fields for the environmental monitoring and protection agencies, crime scene investigations and homeland security.

A broad range of methods were developed for the analysis of explosives such as thin layer chromatography (TLC) [7], infrared spectroscopy (IR) [8], gas chromatography (GC) [9] and liquid chromatography (LC) [10]. Among them, liquid chromatography offers the possibility to analyze directly and simultaneously a large variety of analytes without the risk of degradation of ther-

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mally instable compounds which can occur during GC analysis. The United States Environmental Protection Agency (USEPA) offers a standard method based on HPLC with a UV detector for the quantification of a defined list of nitroaromatics and nitroamines explosives (Method 8330B). But this method has several drawbacks such as poor sensitivity and a lack of selectivity which make the quantification at trace levels in complex matrices quite challenging. More recently, the analytical performances were improved with the introduction of ultra-high performance liquid chromatography (UHPLC) which permits the use of columns with a particle size of less than 2 µm [11].

A good alternative to UV detection for energetic compounds is the use of mass spectrometry (MS) or tandem mass spectrometry (MS/MS) resulting in a dramatic increase in both sensitivity and selectivity, especially when MRM mode is employed [12].

For explosives, the most commonly used ionization methods rely on atmospheric pressure ion sources (API) such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) which are easily coupled to an HPLC or UPLC system. ESI has been used for the quantification of nitropentaerytritol (PETN), hexogen (RDX) or octogen (HMX) [13] for which an excellent sensitivity can be obtained. On the other hand, detection limits are higher for other common nitroaromatic explosives such as trinitrotoluene (TNT), dinitrotoluene (DNT) or trinitrobenzene (TNB). Besides, ESI suffers of a lack of reliability due to the matrix effects which can occurs when performing the direct analysis of real samples. On the contrary, APCI is recognized to be very reliable, with less variation due to matrix effects than ESI. Thus, the organic solvent composition, pH and ionic strength of the samples are parameters that can be modified with very little modifications in signal intensity. Besides, APCI allows the analysis of several nitroaromatic compounds with a lower limit of detection compared to ESI [14].

Assessing the pollution of a contaminated area requires detecting and characterizing the types of explosives and their degradation products in soil, groundwater and surface water. This can be done either by collecting samples on site and bringing them to the laboratory for analysis or by directly extracting the explosives on-site in the contaminated area using an adequate sample preparation technique for enrichment of the targeted compounds. The sensitivity and reliability of the analysis can be both dramatically increased by performing a sample preparation method. Indeed, various extractions methods targeting energetic compounds have been explored, such as column preconcentration [15], dispersive liquid liquid micro extraction [16], or solid phase extraction (SPE). Normal phase SPE [17], reversed phase SPE [18] and online SPE [19] were developed and successfully used to remove matrix interferences and increase the signal to noise ratio. In SPE, a broad range of stationary phases can be used, but the best results for explosives were obtained with polymeric polar phases compared to octadecyl-bonded silica [20]. Despite the good performance obtained, SPE is time consuming and may involve the use of a large quantity of chemicals for both the cleaning and the elution steps. Besides, automation of SPE is quite difficult and expensive. On the other hand, solid phase micro extraction (SPME) can be easily automated with a relative lower cost than automated SPE. Good results were obtained for the thermally stable nitroaromatic compounds such as nitrotoluene (NT), dinitrotoluene (DNT), dinitrobenzene (DNB) or trinitrotoluene (TNT), when SPME was associated with a thermodesoprtion-gas chromatographic analysis [21]. This method is, however, limited to thermally stable and volatile compounds.

An increasingly popular method in analytical chemistry is stir-bar sorptive extraction (SBSE), a method introduced by Baltussen et al. [22], where the sample is stirred with a glass enclosed magnetic stir bar, called a Twister™ coated with a layer of polydimethylsiloxane (PDMS) into which the analytes partition. This technique is less time consuming than liquid/liquid extraction or

classical SPE. Besides, automation of conditioning is possible, and the cleaning of the stir bar is easier than in classical SPE. In SBSE, the extraction can be carried out by immersion of the stir bar into the aqueous sample or in the headspace. The amount of sorptive phase (PDMS) in SBSE is greater relative to SPME, resulting in a lower phase ratio (volume of water/volume of coating), ultimately increasing analyte recoveries and thereby enhancing sensitivity. Although SBSE has been successfully applied for a wide range of analytes from environmental, biological and food samples [23–26], this technique has seldom been explored for the analysis of energetic compounds. Lokhnauth and Snow [27] have tested the SBSE coupled to an analysis by ion mobility but their study focused on the extraction of TNT and RDX with PDMS stir bars.

To optimize an SBSE procedure, many parameters have to be tested and optimized for both the extraction and desorption steps. Indeed, the influence of the chemical nature of the stir bar polymeric sorbent, the sample's pH, the addition of organic modifiers, or salt addition, are among the most studied experimental parameters. Besides, the desorption (thermal or liquid) involve an additional optimization, increasing the number of factors to be tested, and consequently, the number of experiments to perform. Most of the SBSE methods developed use a "one variable at a time" approach to optimize their process [28–32]. The disadvantage of this approach is the impossibility to determine the interaction among the different factors tested. A few studies have reported the use of chemometric approaches, such as design of experiment methodologies [25,33–40]. This approach, unlike the "one variable at a time" allows to determine the interaction between factors as well as the quadratic effects, depending of the type of experimental design chosen. It should be noted that effect of SBSE parameters are strongly molecule dependent. That is the reason why they have to be tested for each new development.

The aim of this paper is to develop and optimize a new SBSE extraction method followed by liquid desorption, for explosive analysis by liquid chromatography–tandem mass spectrometry. The extraction and liquid desorption steps were optimized separately by using Doehlert and full factorial design respectively; before performing a kinetic study of SBSE explosive extraction under optimized conditions. The use of an experimental design methodology allows the complete study of 10 experimental parameters on 8 analytes including nitro aromatics, nitric esters and amino-nitro-aromatic compounds. Even if this study focusses on a multi-compounds method, large amount of information regarding the effects of matrix modifiers, desorption parameters and extraction times can be used for other specific developments such as other explosives with similar structure, or other degradation products.

2. Material and methods

2.1. Chemicals and reagents

A commercial mixture of 2-amino-4,6-dinitrotoluene (2A46DNT), 4amino-2,6-dinitrotoluene (4A26DNT), 1,3-dinitrobenzene (13DNB), 2,4-dinitrotoluene (24DNT), 2,6-dinitrotoluene (26DNT), HMX, RDX, Tetryl, 1,3,5-trinitrotoluene (TNT) and 1,3,5-trinitrobenzene (TNB) at 1000 µg/mL in methanol/acetonitrile (1:1) was purchased from AccuStandard Europe. Pentrite (PETN) at 1000 µg/mL in methanol was also purchased as a separate standard from AccuStandard Europe. All solutions were kept in the dark and refrigerated.

Methanol was HPLC-grade and obtained from Fisher Scientific (Illkirch, France). Water was purified with a Millipore MilliQ-10 system. Ammonium formate added to the aqueous mobile phase was 97% of purity and obtained from Sigma–Aldrich (Saint Quentin Fallavier, France).