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An evaluation of solid phase microextraction for aliphatic amines using derivatization with 9-fluorenylmethyl chloroformate and liquid chromatography

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Abstract

The reliability of SPME combined with a chemical reaction for the analysis of short-chain aliphatic amines by liquid chromatography has been investigated. Different options to couple SPME and derivatization have been tested and compared: (i) derivatization of the analytes in solution followed by the extraction of the derivatives, (ii) extraction of the analytes and subsequent derivatization by immersing the SPME fibre onto a solution of the reagent, and (iii) extraction/derivatization of the analytes using fibres previously coated with the reagent. Methylamine (MA), dimethylamine (DMA) and trimethylamine (TMA) have been selected as a model of primary, secondary and tertiary amines, respectively. The analytes have been derivatized with the fluorogenic reagent 9-fluorenylmethyl chloroformate (FMOC), and the fibre coating was Carbowax-templated resin (CW-TR). The employment of fibres coated with FMOC to extract and derivatize the analytes was the best option, as compared with the other approaches tested the sensitivity was considerably improved. On the basis of these studies, a new procedure for the determination of MA, DMA and TMA in water is presented. To demonstrate the utility of the proposed conditions data on linearity, accuracy, repeatability and sensitivity are given. Results of the determination of the amines in tap, river and waste water are also presented.

Keywords: SPME; Derivatization; Amines; FMOC; Liquid chromatography

1. Introduction

Solid-phase microextraction (SPME) has emerged as an attractive alternative to conventional sample preparation procedures, and there is a growing interest in this technique from many fields (environmental, clinical, toxicological, forensic, food analysis, natural and industrial products). This is because SPME integrates sampling, analyte concentration and sample introduction in a single process. Moreover, it can be coupled to gas chromatography (GC) and to liquid chromatography (LC), as well as to other instruments. Additional advantages over conventional procedures such as liquid–liquid extraction (LLE) or solid-phase extraction (SPE) are the reduction on the consumption of organic solvents, the possibility of effecting on-site sampling, or the compatibility with portable equipments. Despite of its numerous advantages, SPME also presents some drawbacks. It is generally recognised that the low sensitivity attainable is an important limitation compared with LLE or SPE based procedures. The reason is that owing to the small dimensions of the fibre coating the amount of analyte that can be extracted from the samples is low [1,2]. This is particularly important in the case of polar analytes due to their low affinities for common fibre coatings.

In order to enhance the sensitivity in SPME two main alternatives have been explored: the development of new fibre coatings, and the transformation of the analytes via a chemical reaction into compounds more amenable for their extraction. The utility of coupling derivatization to SPME has been extensively documented for a wide variety of matrices and analytes. However, a vast majority of the described applications deal with the derivatization of solutes prior to GC, as reflected in published reviews covering this topic [3,4]. In contrast, the combination of SPME and a chemical reaction for LC has received much limited attention, in spite of the fact that many LC determi-

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nations require prior derivatization of the analytes. A typical example is the determination of short-chain aliphatic amines in water. The analysis of these compounds generally entails preconcentration followed by a chromatographic separation. This is a difficult task because short-chain aliphatic amines present high polarity, water solubility and volatility. Moreover, they are rather insensitive towards common LC detectors. For those reasons, a vast majority of the LC assays proposed for aliphatic amines incorporate a chemical derivatization to transform the analytes into derivatives that can be more easily isolated, resolved and detected [5–9].

The combination of SPME and chemical derivatization for aliphatic amines was firstly reported by Pawliszyn et al. [10]. The authors demonstrated that the transformation of the analytes into less polar derivatives reduced considerably the limits of detection (LOD) over direct SPME. Derivatization has been also used to increase the extraction efficiency with fibre coatings specially synthetised for short-chain aliphatic amines [11]. In both studies GC was used to separate and quantify the derivatized amines. We have recently demonstrated the utility of combining SPME with a chemical derivatization for the determination of methylamine (MA) in water prior to LC [12]. For this purpose, CW-TR coated SPME fibres were successively immersed into the samples and into a solution of the reagent, 9-fluorenylmethyl chloroformate (FMOC). The same extraction/derivatization scheme was adapted to reactions involving the sequential use of two derivatization agents [13]. However, other possibilities available for combining SPME and derivatization remain unexplored.

In the present work, we evaluated and compared different options to couple derivatization and SPME for short-chain aliphatic amines using LC: (i) solution derivatization followed by extraction of the derivatives, (ii) extraction of the analyte and subsequent derivatization by immersing the fibre onto a solution of the reagent, and (iii) extraction of the analytes using fibres previously coated with the reagent. FMOC was selected as derivatizing agent because, unlike most of the reagents available for compounds containing amino groups, it is also capable of reacting with tertiary amines [14]. MA, dimethylamine (DMA) and trimethylamine (TMA) were selected as a model of primary, secondary and tertiary amines, respectively. In order to achieve a better understanding of the processes involved, the results were compared with those obtained by processing directly samples subjected to a conventional solution derivatization.

2. Experimental

2.1. Apparatus and chromatographic conditions

The chromatographic system consisted of a quaternary pump (Hewlett-Packard 1050 Series, Palo Alto, CA, USA), a SPME–HPLC interface (Supelco, Bellefonte, PA, USA) and a fluorescence detector (Hewlett-Packard, 1050 Series). The detector was coupled to a data system (Hewlett-Packard, HPLC Chem Station) for data acquisition and calculation. The excitation and emission wavelengths were 264 and 313 nm, respectively. A SPME assembly with replaceable extraction fibres coated with Carbowax-templated resin, (CW-TR, 50 μm, Supelco) was used for extraction and/or derivatization.

A Clifton water bath equipped with a thermostat (Nickel Electro LTD, Avon, England) was used to perform derivatization at temperatures other than ambient temperature.

2.2. Reagents and solutions

All the reagents were of analytical grade. Methylamine, dimethylamine, and trimethylamine were obtained from Sigma (St. Louis, MO, USA), and 9-fluorenylmethyl chloroformate was purchased from Aldrich (Steinheim, Germany). Acetonitrile was of HPLC grade (Scharlau, Barcelona, Spain). Sodium hydroxide and boric acid were obtained from Panreac (Barcelona, Spain).

Stock standard solutions of the amines (1.0 g/L) were prepared in water. Working solutions of the analytes were prepared by dilution of the stock solutions with water. The pH of the samples was adjusted to 10.0 by adding 0.5 M sodium hydroxide. Water was deionized and filtered through 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain). All solutions were stored in the dark at 2 °C.

The FMOC solutions were prepared daily by dissolving the pure compound in acetonitrile. The 0.05 M borate buffer used in derivatizations was prepared by dissolving the appropriate amount of boric acid in water. Then the pH was adjusted to 9.0 with 0.5 M NaOH.

2.3. Chromatographic conditions

A LiChrospher 100 RP_{18}, 125 mm × 4 mm i.d. column (Merck, Darmstadt, Germany) was the analytical column. The mobile-phase was a mixture of acetonitrile–water in gradient elution mode. The mobile-phase flow rate was 1 mL/min. In SPME assays a precolumn and a high-pressure six-port valve (Hewlett-Packard) were inserted between the SPME–HPLC interface and the analytical column to effect peak compression [12]. The precolumn (20 mm × 2.1 mm i.d.) was dry-packed with a Hypersil C_{18}, 30 μm, stationary phase. Initially, the precolumn and the analytical column were connected and equilibrated with water. During the desorption of the analytes in the SPME–HPLC interface the switching valve was rotated, so the eluent emerging from the precolumn (water) was sent to waste. The chromatographic run was started when the SPME–HPLC interface was activated to send the FMOC derivatives from the interface to the precolumn. At 0.5 min, the six-port valve was again rotated so precolumn and the analytical column were connected. Meanwhile, the acetonitrile content in the mobile phase was linearly increased from 0 to 60% (v/v) at 2.5 min, and then to 70% (v/v) at 9 min, and to 100% at 17 min (in such a way, both the precolumn and the analytical column were cleaned at the end of each assay).

All solvents were filtered through 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain) and degassed with helium before use.