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Multiple headspace solid-phase microextraction of ethyl carbamate from different alcoholic beverages employing drying agent based matrix modification

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ABSTRACT

Multiple headspace solid-phase microextraction (MHS-SPME) combined with gas chromatography-nitrogen phosphorus detector is proposed to determine the toxic contaminant ethyl carbamate (EC) in various alcoholic beverages after matrix modification. The remarkable feature of this method is that matrix effect, which commonly appears in SPME-based analysis, is avoided by determining the total amount of the analyte in the sample. To increase the sensitivity of the method, a novel polyethylene glycol/hydroxy-terminated silicone oil fiber was developed by sol–gel technique and applied for the analysis. Owing to the high polarity and hydrophilia of EC, an important problem still remains because the adsorption by sample matrix causes low transport of EC to the headspace and thus invalidates MHS-SPME for quantification. Mixing with anhydrous sodium sulphate, the sensitivity of the method can be improved. A Taguchi's L_{16} (4^5) orthogonal array design was employed to evaluate potentially significant factors and screen the optimum conditions for MHS-SPME of EC. Under the optimized conditions, limit of detection of 0.034 mg L^{-1} was obtained. Relative standard deviation of replicate samples ($n = 6$) was 2.19%. The proposed method was linear in the range of 0.04–100 mg L^{-1}, and the coefficient of determination was 0.9997. The method was used to determine EC in various alcoholic beverages. The concentrations obtained were compared with those obtained by standard addition method and no statistically significant differences were observed.

1. Introduction

The presence of ethyl carbamate (EC) in alcoholic beverages and fermented foods is a problem that has caused public health concern in the past few years as EC was re-classified as a carcinogen (Group 2A) by the International Agency for Research on Cancer in 2007 [1]. Considering that alcoholic beverages represent the highest part of EC intakes, several countries have established limitations on their levels [2]. This has forced the scientific community to develop analytical procedures that can determine not only the presence of EC in different alcoholic beverage samples but also their concentrations with a good accuracy.

Chromatography has become an important tool in the quantitative analysis of EC in various matrices. An important problem in the method development by chromatographic techniques is the possible occurrence of matrix effects, especially for complex samples. In most cases, matrix effect is considered to be a suppression or enhancement of the analyte response due to the matrix constituents. In general, there are two forms of matrix effect by chromatographic techniques. The first one is caused by co-eluting compounds, which show similar chromatographic behavior (i.e. retention time). It can be controlled by the improvement of the chromatographic separation and specific detectors, for which multidimensional chromatography [3] and tandem mass spectrometric detector [4] emerge as the powerful analytical techniques. The other is caused by co-existing components in sample matrix, which affect the extraction of the analyte and lead to a poor recovery. This is often solved by improvements in sample pretreatment procedures. Liquid–liquid extraction (LLE) [5,6] and solid phase extraction (SPE) [4,7,8] are often used for the determination of EC in alcoholic beverages. Nevertheless, these techniques require extensive organic solvents and are time-consuming. Solid-phase microextraction (SPME), a versatile solvent-free extraction technique, represents a good alternative to the aforementioned techniques. It has been used to extract EC in beers [9], wines [3,9,10], stone-fruit spirits [11] and grape brandies [9].

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Unlike traditional sample preparation methods, such as LLE, SPE and Soxhlet extraction, SPME is a non-exhaustive extraction technique in which only a small portion of the target analyte is removed from the sample matrix. The different components and characteristics of the matrices cause considerable differences in the partition coefficients and release rates of the same analytes. It implies that a careful calibration is necessary to compensate for the matrix-effect error. Different measures were taken for headspace SPME (HS-SPME) of EC in alcoholic beverages. Zhang and Zhang [9] found that using an internal standard could eliminate the effect of ethanol. But the effects caused by the other compositions in different types of alcoholic beverages were not mentioned when the method was applied to real samples. Apart from adding a deuterated internal standard, Lachenmeier et al. [11] introduced a sample preparation by diluting the stone-fruit spirits samples to disrupt the ethanol micelles and to reduce the competitive influence. To a great extent, the internal standard method can compensate for the effect of complicated matrix, but cannot avoid it thoroughly, and systematic errors may occur in the quantification step. And also sometimes isotopically labelled standard substances may cause the matrix effect as well [12]. Besides, the external standard calibration with model wines [3] has also been proposed to remove the effects of complex samples. However, it cannot represent the real matrix absolutely.

Multiple HS-SPME (MHS-SPME) is proposed as a suitable alternative in order to avoid the matrix effect [13]. The quantitative approach of MHS-SPME is theoretically different from that of HS-SPME. This technique involves sampling repeatedly the same vial by HS-SPME. When a portion of the analytes in the headspace is removed in the first extraction, the equilibrium between the analytes in the sample and those in the headspace is disturbed. As the sample is intended to re-equilibrate, more analytes migrate from the sample into the headspace. The concentrations in the two phases will now be smaller than those during the first extraction, but the ratios of these concentrations in the two phases will be the same. The second extraction and analysis, thus, results in a smaller peak. By continuing this procedure it is possible to extract all the analytes from the sample. If carried out ad infinitum, all of the peak areas are summed up to get the total peak area, which corresponds to the total amount of the analyte in the sample. The use of MHS-SPME enables a complete recovery of the target compounds and therefore, the matrix effect is avoided by the exhaustive extraction. As the logarithms of the various area values from the consecutive analyses are plotted versus the number of extractions in a linear scale under certain circumstances, the total area value can be obtained by regression calculation from the areas obtained in only a few extraction steps [13]. In this way, the total area (A_T) can be calculated using the following mathematical Eq. (1) when the extraction is not exhaustive, or directly calculated as the sum of the areas of each individual extraction when it is exhaustive:

$$A_T = \sum_{i=1}^{\infty} A_i = \frac{A_1}{1-\beta} \quad (1)$$

where A_1 is the peak area of the first extraction and β (constant) is calculated from the linear regression of the logarithms of the individual peak area:

$$\ln\ Ai = \ln\ A1 + (i-1)\ln\ \beta \quad (2)$$

where A_i is the relative peak area obtained in the ith extraction.

As described in the literatures, MHS-SPME has been applied to the determination of volatile compounds in different types of matrices including packaging materials [13], soils [14], tomato [15], oils [16] and wines [17–19]. To evaluate the applicability of the aforementioned MHS-SPME method, the results are usually compared with those obtained by standard addition method, and the concentrations gained by both methods for the analytes are statistically equivalent. However, MHS-SPME has certain drawbacks such as increased analysis time compared with one-step SPME. There is a way to reduce analysis time, to perform MHS-SPME under a non-equilibrium situation. The theoretical principals of MHS-SPME under both equilibrium [13] and non-equilibrium [20] conditions have been presented. In our study, a fast MHS-SPME method is developed under a non-equilibrium situation.

Although MHS-SPME would be a good approach in principle, the usefulness of this method is limited. It may be difficult to achieve the exponential decay in peak area for all analytes because some interferences exist. The decay is characteristic for each analyte and depends on sample matrix and extraction conditions [15]. For this method to be effective, analytes must be released easily from their matrix into the headspace. For volatiles, the main challenge is the possible trapping and adsorption of analytes on the micro-phases of matrix [21], while for semi-volatile analytes low volatility is also a major concern. EC is highly polar and hydrophilic, which is an important limiting factor in MHS-SPME. EC is easily soluble in water and alcohol. It is relatively involatile and stable in aqueous solutions. The most commonly used matrix-modification techniques in the case of liquid samples, such as NaCl addition [11,22–25], temperature [22–25] and pH [9,11,25] adjustment, were employed in our preliminary study. But satisfactory results were not achieved yet. It showed that the matrix retained EC strongly in aqueous system, which caused low migration of analyte to the headspace and deviations from linearity of $\ln A_i$ plots. Efforts should be made to reduce the interference of matrix components in the samples.

Additionally, a suitable SPME fiber is needed to provide an appropriate coating-sample distribution coefficient, since in MHS-SPME it is essential to extract a significant amount of analyte in relation to the total amount in order to observe an exponential decay of peak areas versus the number of extractions. The carbowax/divinylbenzene (CW/DVB) fiber was usually employed for the headspace extraction of EC in alcoholic beverages [9–11]. However, it is not commercially available because of the solvent instability, swelling and stripping of the coating [26]. Therefore, the development of effective extraction coatings is in urgent need nowadays.

The aim of this study was to develop a simple, sensitive and reliable method for the analysis of EC in different alcoholic beverages. MHS-SPME was employed to avoid matrix effect from different samples, and additionally, drying agent based matrix modification was introduced to reduce the interference of water and enhance EC amount in the headspace. This approach has not been used in SPME for this purpose. To increase the sensitivity of the method, a new SPME coating made from polyethylene glycol (PEG) and hydroxy-terminated silicone oil (OH-TSO) was developed with sol–gel technique and applied for the analysis of EC followed by gas chromatography-nitrogen phosphorus detector (GC-NPD).

2. Experimental

2.1. Chemicals and standard solutions

EC (>97%) was purchased from J&K Chemical Ltd. (New Jersey, USA). PEG-20M (average molar mass ranging from 14,000 to 16,000 g mol^{-1}), sodium hydroxide (NaOH), tartaric acid, sodium chloride (NaCl), anhydrous sodium sulphate (Na_2SO_4), ethanol and acetone were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), which were all analytical-reagent grade. OH-TSO, 3-(2-cyclooxypropoxyl) propyltrimethoxysilane (KH-560), tetraethoxysilane (TEOS), and poly (methylhydrosiloxane) (PMHS) were purchased from Wuhan University Silicone New Material Co., Ltd. (Wuhan, China). Trifluoroacetic acid (TFA) was purchased from Shanghai Chemical Factory, China. Ultrapure water