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## Rapid and sensitive methodology for determination of ethyl carbamate in fortified wines using microextraction by packed sorbent and gas chromatography with mass spectrometric detection<sup>☆</sup>

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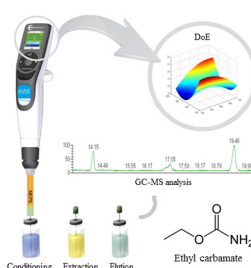
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### HIGHLIGHTS

- MEPS was first used to quantify ethyl carbamate in fortified wines.
- The extraction was optimized (DoE) to 300  $\mu\text{L}$  of sample and 100  $\mu\text{L}$  of dichloromethane.
- Good linearity ( $R^2 = 0.9999$ ) and low LOQ ( $4.5 \mu\text{g L}^{-1}$ ) were obtained.
- The method applicability was demonstrated by the analysis of 16 fortified wines.
- MEPS ensured efficiency and effectiveness without using sophisticated equipment.

### GRAPHICAL ABSTRACT



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### ABSTRACT

This work presents a new methodology to quantify ethyl carbamate (EC) in fortified wines. The presented approach combines the microextraction by packed sorbent (MEPS), using a hand-held automated analytical syringe, with one-dimensional gas chromatography coupled with mass spectrometry detection (GC–MS). The performance of different MEPS sorbent materials was tested, namely SIL, C2, C8, C18, and M1. Also, several extraction solvents and the matrix effect were evaluated. Experimental data showed that C8 and dichloromethane were the best sorbent/solvent pair to extract EC. Concerning solvent and sample volumes optimization used in MEPS extraction an experimental design (DoE) was carried out. The best extraction yield was achieved passing 300  $\mu\text{L}$  of sample and 100  $\mu\text{L}$  of dichloromethane. The method validation was performed using a matrix-matched calibration using both sweet and dry fortified wines, to minimize the matrix effect. The proposed methodology presented good linearity ( $R^2 = 0.9999$ ) and high sensitivity, with quite low limits of detection (LOD) and quantification (LOQ),  $1.5 \mu\text{g L}^{-1}$  and  $4.5 \mu\text{g L}^{-1}$ , respectively. The recoveries varied between 97% and 106%, while the method precision (repeatability and reproducibility) was lower than 7%. The applicability of the methodology was confirmed through the analysis of 16 fortified wines, with values ranging between 7.3 and 206  $\mu\text{g L}^{-1}$ . All chromatograms showed good peak resolution, confirming its selectivity. The developed MEPS/GC–MS methodology arises

**Abbreviations:** EC, ethyl carbamate; MEPS, microextraction by packed sorbent; GC–MS, gas chromatography–mass spectrometry; FW, fortified wine; DoE, experimental design; BIN, barrel insert needle; IS, internal standard; ME, matrix effect; LOD, limit of detection; LOQ, limit of quantification.

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as an important tool to quantify EC in fortified wines, combining efficiency and effectiveness, with simpler, faster and affordable analytical procedures that provide great sensitivity without using sophisticated and expensive equipment.

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## 1. Introduction

Ethyl carbamate (EC), also known as urethane, is the ester of carbamic acid ( $\text{H}_2\text{NCOOC}_2\text{H}_5$ ). It is known as a toxic compound and was re-classified in 2007 by the International Agency of Research on Cancer (IARC) as a probably carcinogenic to humans (Group 2A) [1].

EC is formed in small amounts in fermented or heated food, namely in alcoholic beverages, including fortified wines. The EC formation in these foodstuffs is usually associated with storage time and temperature [2]. This compound results from the reaction between ethanol and nitrogenous compounds like urea, citruline, hydrocyanic acid and N-carbamyl compounds [3–5]. One of the most common pathways proposed to explain the development of EC in acid media consists in the reaction of urea with ethanol [6]. The kinetics of this reaction is greatly enhanced by the temperature increase [7,8]. Urea and citruline can be detected in wine and are both derived from the arginine metabolism during the fermentative processes [6,8,9]. Another precursor of EC referenced in the bibliography is hydrogen cyanide derived from cyanogenic glycosides, produced by several plant species, including *Vitis vinifera* L. [10,11]. The formation of EC via cyanide is mostly originated through procedures that include thermal treatments like distillation or baking [6].

The toxicological concerns led Canada to establish by the first time, in 1985, legislation regulating the EC limit values in alcoholic beverages, namely in fortified wines to  $100 \mu\text{g L}^{-1}$ . Other legal limits were also imposed:  $30 \mu\text{g L}^{-1}$  for table wines,  $150 \mu\text{g L}^{-1}$  for distilled spirits,  $200 \mu\text{g L}^{-1}$  for sake and  $400 \mu\text{g L}^{-1}$  for fruit brandies and liqueurs [6,12]. In Europe, only Czech Republic follows the Canadian legislation for fortified wine [12].

The concerns raised by the toxicological aspects of EC together with the low concentration levels ( $\mu\text{g L}^{-1}$ ) found in wines, as well as the occurrence of interferences on detection, has motivated several researchers to develop new methods to determine it in wines. Several extraction and chromatographic techniques have been used, including continuous liquid–liquid extraction (LLE) with Soxhlet apparatus [13], derivatization with 9-xanthidrol followed by high performance liquid chromatography (HPLC) with fluorescence detection [14] and even LLE after derivatization, followed by gas chromatography coupled with mass spectrometry detection (GC–MS) [15]. On the other hand, the reference method set by the International Organization of Vine and Wine (OIV) [17] uses solid phase extraction (SPE) preceding GC–MS quantification [16]. Other methods also make use of SPE, but use gas chromatography with mass spectrometry (MDGC/MS) [18] and liquid chromatography with tandem mass spectrometry (LC–MS/MS) for detection [19]. Most of the methodologies found in the literature to quantify EC use gas chromatography, using LLE [13,20,21] and SPE [16–18,22,23] as extraction techniques. Nevertheless, several efforts have also been done to develop new methodologies to determine EC without using long procedures and hard-working analyses, combining precision to high sensitivity. In this regard, headspace solid phase microextraction (HS–SPME) has been gaining great highlighting [24–26] and alternative methodologies has been proposed using the most recent identification and quantification technology, such as gas chromatography with tandem mass spectrometry detection (GC–MS/MS) [26] and two-dimensional gas chromatography with time-of-flight mass spectrometry ( $\text{GC} \times \text{GC}$ –ToFMS) [25]. Liao

et al. [27] also used an emergent extraction technique, based on ultrasound-assisted emulsification–microextraction (USAEME) to extract EC in alcoholic beverages, but using gas chromatography coupled to triple quadrupole mass spectrometry. However, this kind of technologies is still not accessible to many laboratories.

Recently, microextraction by packed sorbent (MEPS) has also becoming emergent, arising as a feasible and easy-to-use extraction technique. MEPS derives from the miniaturization of the conventional SPE, but with additional advantages: uses small sample and solvent volumes (microliters) and consequently reduces the environmental impact, increases the analysis sensitivity and enables the direct injection into the LC or GC instruments. The small cartridge can be packed or coated with different silica-based polymers: SIL (unmodified silica), C2 (ethyl), C8 (octyl), C18 (octadecyl) and M1 (80% C8 and 20% SCX – strong cation exchanger using sulfonic acid bonded silica), providing selective and suitable sampling conditions [28]. The MEPS technique has been used to determine other compounds of interest for the alcoholic beverages industry [29–31]; however, as far as we know, it has never been applied for the analytical determination of EC.

The aim of this study was the development of a fast, simple and sensitive methodology to quantify EC in fortified wines using MEPS extraction combined with one-dimensional GC–MS equipment, accessible to most laboratories.

## 2. Materials and methods

### 2.1. Chemicals and samples

Ethyl carbamate (EC) was purchased from Acros Organics (Geel, Belgium), while butyl carbamate (BC), used as an internal standard (IS), was obtained from Sigma–Aldrich (Steinheim, Germany). All standards had a purity grade of more than 97%. Absolute ethanol, >99.8% (GC), was purchased from Sigma–Aldrich (Steinheim, Germany), tartaric acid and methanol from Panreac (Barcelona, Spain) while acetonitrile, ethyl acetate and dichloromethane were from Fisher Scientific (Leicestershire, UK). Ultra-pure water (18 M $\Omega$ ) was prepared by the Simplicity<sup>®</sup>UV ultrapure water (type 1) apparatus from Millipore (Milford, MA, USA).

EC and BC stock solutions of  $1 \text{ g L}^{-1}$  were prepared by dissolving appropriate amounts of each compound in ultra-pure water. In order to obtain the matrix-matched calibration solutions, suitable dilutions of the stock solutions were prepared with ultra-pure water, to obtain the intermediate solutions of  $50 \text{ mg L}^{-1}$  in EC and  $10 \text{ mg L}^{-1}$  in BC, which were then used to spike dry and sweet fortified young wines. Each calibration point was extracted in triplicate, within the validation range 5–400  $\mu\text{g L}^{-1}$ .

The sweet and dry fortified wines used to perform the matrix-matched calibrations were obtained from *V. vinifera* L. white varieties and were absent of quantifiable amounts of EC and BC. Regarding the application sample set, 16 fortified wines, aged up to 36 years old and with ethanol contents between 18% and 20% were analyzed using the developed methodology.

### 2.2. Apparatus and chromatographic conditions

eVol<sup>®</sup> MEPS<sup>™</sup> hand-held automated analytical syringe (SGE Analytical Science, Australia) of 500  $\mu\text{L}$  was used and MEPS barrel

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