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Short communication

Determination of ethyl carbamate in pálinka spirits by liquid chromatography–electrospray tandem mass spectrometry after derivatization

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ABSTRACT

A robust and selective method for the determination of ethyl carbamate in double-distilled fruit brandies ("pálinka" spirits) by HPLC-ESI-MS/MS is described in the study. The approach is based on the combination of xanthydrol derivatization and the multiple reaction monitoring (MRM) of xanthyl-ethyl carbamate. Interestingly, this compound could only be ionized with adequate intensity through $[M+Na]^+$ adduct formation, similarly to some of the carbamate derived pesticides, as investigated with the help of synthesizing the commercially unavailable standard. 20 traditional spirit samples originating from 14 fruit species were analyzed with the developed method, showing high selectivity and fruit-dependent sensitivity, thus requiring standard addition for quantification purposes. The concentration range of ethyl carbamate in the spirit samples was between the detection limit (0.003 mg L^{-1}) and 2.6 mg L^{-1}. The study indicated that besides the usually high ethyl carbamate containing, stone fruit derived spirits namely, plum (*Prunus domestica* L.) and sour cherry (*Prunus cerasus* L.), spirit samples of quince (*Cydonia oblonga* Mill.), a non-stone fruit species, showed relatively high ethyl carbamate levels close to or exceeding the actual European recommendation of 1 mg L^{-1}. As quince is not known to contain either high amounts of cyanogenic glucoside or N-carbamyl-amino acids, this phenomenon cannot be actually referred to genuine inner parameters of this fruit.

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1. Introduction

Concentration of ethyl carbamate, a toxic compound that may occur in fermented foods and beverages due to natural biochemical processes (Nout, 1994; Weber & Sharypov, 2009) has been regulated and limited in several countries such as Germany, USA, Canada, France and the Czech Republic (Lachenmeier, 2005). According to the recent commission recommendation of the European Food Safety Authority (EFSA) issued in March 2010 (EUR-Lex, 2010), special attention should be taken to usual and marc spirits distilled from stone fruits as this kind of drinks may contain the highest relative amount of ethyl carbamate, exceeding sometimes 1 mg L^{-1}. The document highlights also that EU countries should regularly monitor the concentration of ethyl carbamate and report the arising data.

This recommendation has increased the need for validated and robust analytical approaches for the determination of ethyl carbamate in spirits that can couple highly selective methods with high sample throughput and uncomplicated sample preparation techniques. From the latter point of view, the derivatization of ethyl carbamate with xanthydrol in spirit samples (Fig. 1), described by Giachetti, Assandri, and Zanolo (1991) and Herbert, Santos, Bastos, Barros, and Alves, (2002), provides an effective way of decreasing matrix effects prior to any kind of detection process (de Melo Abreu, Alves, Oliveira, & Herbert, 2005). However, the proposed analytical method after xanthydrol derivatization, the HPLC-FD technique often reveals several matrix constituents that also possess high FD response factor at the usual 233 nm/600 nm excitation/emission UV/VIS ranges, as presented by Madrera and Valles in cider spirit samples (Madrera & Valles, 2009). The direct HPLC-ESI-MS/MS determination of ethyl carbamate, developed and applied first for the analysis soy sauce samples by Park et al. (2007) featured high sensitivity and recovery but indicated some interferences on the selected MRM transition. Clearly, the advantage of a derivatization step that is selective for carbamate- and urea-related compounds should be completed with a more selective hyphenated analytical technique.

The goal of our study was to develop a selective and robust HPLC-ESI-MS/MS method to quantify ethyl carbamate, based on the xanthydrol derivatization technique. The method was validated and applied for a series of authentic traditional and marc spirits.

2. Materials and methods

2.1. Reagents

Ethyl carbamate (≥99%), 9-xanthydrol, HCl (a.r., 37 m/m%), glacial acetic acid (puriss), acetone (puriss), 1,4-dioxane (puriss) and formic acid (puriss, for use in mass spectrometry) were purchased from the Sigma-Aldrich group (Schnelldorf, Germany). All solvents (ethanol, 1-

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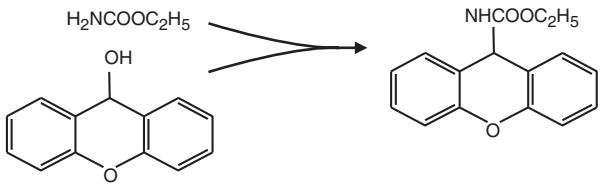

Fig. 1. Derivatization scheme of ethyl carbamate with xanthydrol.

propanol, methanol, and acetonitrile) used were of HPLC gradient grade quality and were obtained from Scharlau (Barcelona, Spain). Ultra pure water was obtained from a Milli-Q system from Millipore (Milford, MA, USA).

2.2. Samples

Ethyl carbamate concentration was quantified for 20 "pálinka" spirits. The pálinka are traditional and EU regulated (EEC No 1576/89) Hungarian or Austrian double-distilled fruit brandies. Samples from various vintages and from various production systems including small-scale (household) and large-scale production were analyzed. The commercially obtained spirit samples were distilled from the following 14 fruit species: cornel (*Cornus mas* L.), quince (*Cydonia oblonga* Mill.), strawberry (*Fragaria X. Ananassa* L.), apple (*Malus domestica* L.), black mulberry (*Morus nigra* L.), apricot (*Prunus armeniaca* L.), cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.), plum (*Prunus domestica* L.), peach (*Prunus persica* L.), pear (*Pyrus communis* L.), red currant (*Ribes rubrum* L.) rosehip (*Rosa rubiginosa* L.), and sorb (*Sorbus domestica* L.). The original alcoholic strength of the spirits (45–62%, V/V) was adjusted to 40% (V/V) with Milli-Q water dilution prior to sample preparation in order not to influence the efficiency of the xanthydrol derivatization process.

2.3. Sample preparation

Xanthyl-ethyl carbamate standard solution was prepared according to Moskalyk and Chatten (1967). Briefly, 2.0 g (0.01 mol) xanthydrol and 1.0 g (0.01 mol) ethyl carbamate were mixed in 15.0 ml of glacial acetic acid and heated at 40 °C for 30 min. The product, 9-xanthyl-ethyl carbamate, crystallized readily and was filtered and washed with 250 ml ice-cold deionized water. The product was recrystallized from 1,4-dioxane:water:acetone (4:4:2) mixture and resulted in white crystals that were washed with ice-cold methanol (25 ml) and air-dried. The yield of the process was 835 mg (28%).

The xanthydrol derivatization process in the spirit samples was a modified approach adapted from Madrera and Valles (2009). Briefly, solutions of 0.1 M xanthydrol dissolved in 1-propanol and 6 M HCl were prepared. The derivatization was carried out in a 10.0 ml volumetric flask. 640 μL of the xanthydrol solution was added to the 8.0 ml spirit sample, afterwards 400 μL HCl solution was added and the volume was made up with methanol. The mixture was homogenized, left at ambient temperature for 30 min, filtered through a 0.22 μm PTFE syringe filter and injected onto the chromatographic system. All samples were measured in triplicate. Quantification was carried out on the basis of a 4-point standard addition, *i.e.*, 100 μL, 200 μL and 500 μL of 24 mg L^{-1} ethyl carbamate were added to the volumetric flask before making up the total volume. To set up method validation parameters 8.0 ml of 40% (V/V) ethanol was applied in the standard addition procedure.

2.4. Analytical procedure

A QTRAP 3200 triple quadrupole-linear ion trap mass spectrometer (Applied Biosystem, Applied Biosystems/Sciex; Foster City, CA, USA) was used either in enhanced product ion (EPI; for optimisation) or multiple reaction monitoring, (MRM, for HPLC analysis) modes. The instrument was equipped with a Turbo V interface and Turbo Ion Spray probe (Applied Biosystems), operating in positive ion mode.

For the flow infusion experiment to screen for the characteristic fragmentation of xanthyl-ethyl carbamate, a 50 mg L^{-1} xanthyl-ethyl carbamate standard (60:40% V/V solution of Milli-Q water and methanol with 0.1% V/V:formic acid) was introduced by means of a syringe pump at a flow rate of 50 μL min^{-1}. The two most intense fragments (m/z 292.1 → 181.1, regarded as quantifier; 292.1 → 152.1, regarded as qualifier) were selected to monitor. The following transition parameters were used: declustering potential (DP) 61 V, entrance potential (EP) 8 V, cell entry potential (CEP) 73 V and 23 V for the m/z 181.1 and 152.1 fragments, respectively, and cell exit potential (CXP) 4 V. The data recorded were processed with Applied Biosystems/MDS-SCIEX Analyst QS software (Frankfurt, Germany).

The HPLC-ESI-MS coupling was achieved by using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA). An Agilent Zorbax XDB-C_{18} reverse phase column (4.6 mm × 150 mm × 5 μm) was eluted in gradient mode. Solution "A" was water with 0.1 V/V% formic acid, and solution "B" was methanol, introduced at 1000 μL min^{-1} with the following gradient: 0–3 min 50% "B", 3–15 min up to 90% "B", 15–20 min 90% "B", and 20–20.1 min down to 50% "B". The injection volume was 50 μL. The column temperature was set to 30 °C. The optimum settings of the HPLC-ESI-MS/MS coupling were as follows: ion spray voltage: 5500 V; curtain gas (N_2): 25 psi; ion source gas: 50 psi; turbo gas: 10 psi; desolvation temperature: 300 °C; collision activated dissociation gas: 5.0 arbitrary units.

Identification of ethyl carbamate from the spirit samples was based on both MRM transition matching (including MRM transition ratios) and retention time matching of the xanthyl-ethyl carbamate standard. Quantification was calculated by integrating the signal of the 292.1 → 181.1 quantifier MRM transition of xanthyl-ethyl carbamate.

3. Results and discussion

3.1. Optimisation and method validation parameters

Fig. 2 shows the EPI spectrum of xanthyl-ethyl carbamate, along with the proposed structures of the two most intense fragments, m/z 181.1 and 152.1. The $[M+H]^+$ ion of the analyte showed inadequate intensity during the flow injection study in EPI mode; however, the $[M+Na]^+$ adduct ion was significantly more intense and proved to be useful for fragmentation experiments. This phenomenon has been described and exploited in the case of N-methyl-carbamate pesticides

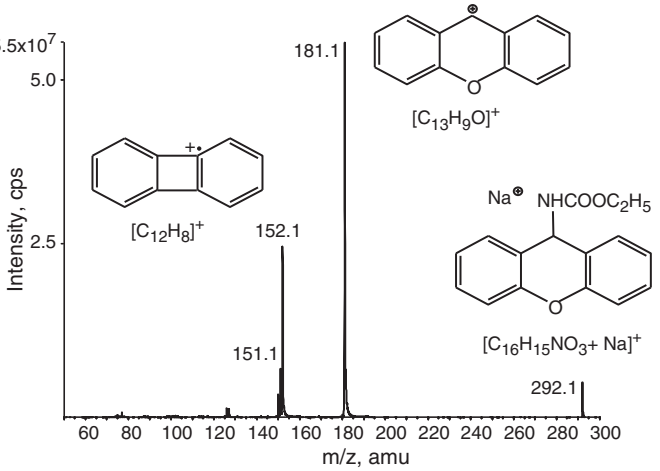

Fig. 2. EPI spectrum of xanthyl-ethyl carbamate. The insets show the structures of the analyte and the proposed fragments.

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