Journal of Food Composition and Analysis 48 (2016) 111–119

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original research article

A new derivatization reagent for determination of biogenic amines in wines

Aneta Jastrzębska*, Anna Piasta, Sylwia Kowalska, Marek Krzemiński, Edward Szłyk

Faculty of Chemistry, Nicolaus Copernicus University in Toruń, 7 Gagarin Str., 87-100 Toruń, Poland

ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form 2 February 2016
Accepted 26 February 2016
Available online 3 March 2016

Keywords:
1-Fluoro-2-nitro-4-(trifluoromethyl) benzene
Biogenic amines
derivatization
Chromatographic determination
Wine samples
Food analysis
Food composition

ABSTRACT

Fermented beverages are the main source of biogenic amines in our diet. Their levels are very difficult to minimize and important for the control of wine quality. Some of them are harmful to human health and methods of their analysis are still developed. In this paper, a new reagent, 1-fluoro-2-nitro-4-(trifluoromethyl)benzene, for precolumn derivatization of selected biogenic amines, and their chromatographic determination is described. The derivatives of histamine, tryptamine, tyramine and phenylethylamine were synthesized in high purity and characterized by ^{1}H, ^{13}C and ^{19}F NMR. A convenient procedure based on the 1-fluoro-2-nitro-4-(trifluoromethyl)benzene has been developed for the determination of these amines in wine samples. The coefficient of variation, $<2.5\%$ for all samples, the recovery $\approx 100\%$, demonstrate repeatability and accuracy of procedure. It is convenient, shows lower solvent consumption, and takes less time in comparison to previously described methods.

1. Introduction

The main biogenic amines present in wines are putrescine, histamine, tyramine and cadaverine, followed by phenylethylamine, spermidine, spermine, agmatine and tryptamine. They are formed by decarboxylation of amino acid with the substrate-specific enzymes of yeast or spoilage bacteria, such as *Lactobacillus*, *Pediococcus,* and *Leuconostoc* species, and by amination and/or transamination of aldehydes and ketones (Wang et al., 2014). The first source of these compounds in wines are grape berries, next fermentation processes, ageing or storage when wine is exposed to the activity of decarboxylase positive microorganisms. It should be emphasized that, agricultural practices and procedures of wine-making cause the appearance of a significant BAs content in wines (Ancín-Azpilicueta et al., 2008; Tuberoso et al., 2015). Apart from the primary metabolic products and many flavour compounds released during fermentations, some microorganisms produce secondary metabolic products such as biogenic amines. Guo et al. (2015) reviewed factors affecting the presence of amines in wines and pointed out oenological factors and vintage, pH value, vinification techniques and ageing of lees, as the most important. It is generally agreed that the concentration of BAs is lower at the end of the alcoholic fermentation and increases mainly during malolactic fermentation. According to Tuberoso et al. (2015), the interaction between ethanol (a monoamine oxidase inhibitor) and amines seems to be synergistic. It should be noted, that most of bioactive amines, consumed in large amounts, represent a health hazard when the mechanism for their catabolism is impaired by disease, pharmacological agents or genetically (Rodriguez et al., 2014).

The European Food Safety Authority confirmed histamine and tyramine as the most toxic and particularly relevant for food safety (Tuberoso et al., 2015) and the products with high contents of BAs may be harmful on susceptible individuals. The levels of BAs can be an important quality and safety indicator of safe storage method of wine products and their wholesomeness. For these reasons, determination of biogenic amines is important for the quality control of wines. Unfortunately, BAs quantification is still problematic due to their low concentration in wine samples and sample complexity, strong polarity, the lack of BAs chromophores and presence of potentially interfering structurally similar substances. Notwithstanding, HPLC is by far the most frequently reported technique for the determination of these compounds in

Abbreviations: BAs, biogenic amines; BAs-NBA, FNBT derivatives of biogenic amines; CNBF, 2-chloro-1,3-dinitro-5-(trifluoromethyl)-benzene); FNBT, 1-fluoro-2-nitro-4-(trifluoromethyl)benzene; Him, histamine; Him-NFA, *N*-(2-(1H-imidazol-4-yl)ethyl)-2-nitro-4-(trifluoromethyl)aniline; Phe, phenethylamine; Phe-NFA, 2-nitro-N-phenethyl-4-(trifluoromethyl)aniline); Trp, tryptamine; Trp-NFA, *N*-(2-(1H-indol-3-yl)ethyl)-2-nitro-4-(trifluoromethyl)aniline; Tyr, tyramine; Tyr-NFA, 4-(2-(2-nitro-4-(trifluoromethyl)phenylamino)ethyl)phenol.

* Corresponding author. Fax: +48 566542477.
E-mail address: aj@chem.uni.torun.pl (A. Jastrzębska).

http://dx.doi.org/10.1016/j.jfca.2016.02.012
0889-1575/

wine samples (Guo et al., 2015). Due to the structural characteristics of BAs, their derivatization is usually needed and typical reagents for the amino group include *o*-phthaldialdehyde (OPA), 9-fluorenylmethylchloroformate, 2,4,6-trinitrobenzenesulfonic acid (TNBS), diethyl ethoxymethylenemalonate (DEEMM), 4-fluoro-3-dinitro-fluoromethylbenzene and dabsyl-chloride (DABS-Cl) (Guo et al., 2015; Gómez-Alonso et al., 2007; Hernández-Cassou and Saurina, 2011; Płotka-Wasylka et al., 2015; Wang et al., 2014) It should be noted, that International Organization of Vine and Wine (OIV) proposed derivatization with OPA and DEEMM for biogenic amines determination in musts and wine using fluorimetric (OIV-MA-AS315-18) and spectrophotometric (OIV-MA-AS315-26) detection methods (http://www.oiv.int/oiv/info/enmethodesinternationalesvin#autres). However, their application is often affected by restrictions or disadvantages, e.g. the reaction with dansyl-chloride is slow and not specific, *o*-phthaldialdehyde reacts only with primary amines, 1,2-naphthoquinone-4-sulphonate reacts at high pH and high temperature (Guo et al., 2015). Hernández-Cassou and Saurina (2011) reviewed the derivatization procedures for the determination of BAs in wines and special attention was paid to 1,2-naphthoquinone-4-sulfonate as a labelling agent. The authors (Hernández-Cassou and Saurina, 2011) suggested, that one of the most important factors of satisfactory quantification is the sample preparation and extraction of derivatives prior to analysis. The latter allows separation of BAs derivatives from matrix components and the reagent excess.

However, the difficulty in developing a derivatization method resides in choosing the adequate reagent, which fulfils the following criteria: method of analysis, selectivity, compatibility with the required reaction conditions, and possibility of working in the precolumn mode. This is particularly important for analysis of wine samples characterized by high amounts of amino acids and organic acids (Hernández-Cassou and Saurina, 2011). Therefore, elaboration of the appropriate derivatization procedures permitting chromatographic separation with satisfactory selectivity and accuracy, is still relevant.

In our previous paper, we described a procedure for the determination of selected BAs based on the derivatization with 2-chloro-1,3-dinitro-5-(trifluoromethyl)-benzene (CNBF) (Piasta et al., 2014). The method ensured quantitative elimination of potential interferences coming out from amino acids or hydrolysis of CNBF, and permitted satisfactory separation and determination of BAs in wine samples. However, the method, despite its advantages, required the time consuming step of sample preparation prior to chromatographic analysis.

In this paper we propose a new, rapid derivatization procedure with 1-fluoro-2-nitro-4-(trifluoromethyl) benzene (FNBT) followed by HPLC analysis. FNBT, similarly do CNBF, readily undergoes nucleophilic aromatic substitution, and it a close analogue of the Sanger reagent (1-fluoro-2,4-dinitro-benzene, FDNB) used in the precolumn derivatization of aminoglycosides in alkaline medium (Isoherranen and Soback, 1999; Šoltés, 1999).

However, the presence of the CF_3 group in FNBT lowers its reactivity towards primary and secondary amines in comparison with the Sanger reagent but also influences hydrophilicity of the product amines (Crampton et al., 2007). Usually, this reagent is used as a starting material for the synthesis of biologically active compounds (Freitag et al., 2011; Gong et al., 2004), and also for derivatization of polyamines, such as putrescine, spermidine and spermine (Lee et al., 2000, 2003; Sohn et al., 2002; Spragg and Hutchings, 1983).

To the best of our knowledge, FNBT has not been used for the BAs precolumn derivatization in food samples and their HPLC determination. Consequently, the main purpose of this paper was the elaboration of a simple and less-time consuming derivatization of histamine (Him), tryptamine (Trp), phenethylamine (Phe), and tyramine (Tyr) derivatization with FNBT. Two procedures for the synthesis of BAs derivatives synthesis (BAs-NFA) with FNBT were developed. The first one was based on the CNBF procedure and included three steps: extraction, synthesis, and second extraction of the obtained derivatives, and the second procedure was shorter and less-time consuming. Both FNBT procedures were applied for the determination of Him, Trp, Phe and Tyr in wine samples by the reversed phase-high performance liquid chromatography (RP-HPLC) and the obtained results were compared with our earlier CNBF procedure (Piasta et al., 2014). The FNBT derivatives were synthesized with high purity and characterized by ^{1}H, ^{13}C, ^{19}F NMR, and single crystal X-ray crystallography (for 2-nitro-*N*-phenethyl-4-(trifluoromethyl) aniline, Phe-NFA). The linearity, repeatability, accuracy, detection and quantification limits for the procedures were calculated. Comparison of obtained mean values for normal population were calculated by a two-sided test whereas precision of the procedures was compared using the *F*-test (Miller and Miller, 2000; Neter et al., 1985).

2. Materials and method

2.1. Reagents and instrumentation

Analytical grade: 1-fluoro-2-nitro-4-(trifluoromethyl) benzene (FNBT), 2-chloro-1,3-dinitro-5-(trifluoromethyl) benzene (CNBF), tyramine hydrochloride (Tyr), histamine dihydrochloride (Him), tryptamine hydrochloride (Trp), phenylethylamine, 99% (Phe), ethanol (absolute), methanol (HPLC grade), *N*,*N*-diisopropylethylamine (DIPEA), hexane 95%, pentane 98%, chloroform-d (99.96% D), trichlorofluoromethane ($CFCl_3$) and tetramethylsilane (TMS) were purchased from Sigma–Aldrich (Poznań, Poland), whereas NaOH, $CH_3COOC_2H_5$, $MgSO_4$ (anhydrous), NaCl from Alchem (Toruń, Poland).

The HPLC system (Liquid Chromatograph LC-20AD, Shimadzu Corporation, Kyoto, Japan), equipped with an autosampler SIL-20AC HT and a photodiode multi-wavelength detector (SPD-M20A Prominence Diode Array Detector) was applied. Analyses were carried out on a Gemini-NX C18 column, (5 μm particle size, 250 × 4.6 mm) at 25 °C. NMR spectra were recorded with a Bruker Avance III 400 MHz spectrometer (Bruker Corporation, Karlsruhe, Germany) at 400 MHz (^{1}H), 100 MHz (^{13}C), and 375 MHz (^{13}F) frequency resonances, at 298 ± 1 K, in $CDCl_3$ 95%, tetramethylsilane (TMS) and trichloro-fluoro-methane were used as standards for ^{1}H, ^{13}C and ^{19}F NMR, respectively. Diffraction data of Phe-NFA were collected on Oxford Sapphire with CCD area detector (CrysAlis and CrysAlis, 2000) at room temperature. The structure was solved by direct methods and refined by full-matrix least-squares techniques on F^2 with SHELXL program (Sheldrick, 2008). The numerical absorption correction was applied for all crystals (RED171 package of programs, Oxford Diffraction, 2000). Heavy atoms were refined with anisotropic thermal displacement parameters. Positions of hydrogen atoms attached to carbon atoms were assigned at calculated positions, whereas position of H7 hydrogen atom attached to nitrogen atom was found from electron density synthesis. All hydrogen atoms were refined with isotropic thermal displacement parameters fixed to a value of 20% higher than those of the corresponding C or N atoms. All figures were prepared in DIAMOND (Brandenburg, 2001) and ORTEP-3 (Farrugia, 1997). CCDC 1412256 contains the supplementary crystallographic data for Phe-NFA. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk.

Prior to the analysis, wine samples were degassed in an ultrasonic cleaner (SB- 5200DTD, Chemland, Starogard Szczeciński, Poland) and centrifuged (laboratory centrifuge MPW-350, MPW MED. INSTRUMENTS, Warsaw, Poland, max speed 9000 rpm, RFC 8693xg, angle 30°, falcon tubes 50 mL). Deionised water