Journal of Chromatography A, xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Determination of 2-Aminoacetophenone in wine by high-performance thin-layer chromatography—fluorescence detection

Nora Horlacher, Wolfgang Schwack*

University of Hohenheim, Institute of Food Chemistry, Garbenstr. 28, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form
27 December 2015
Accepted 28 December 2015
Available online xxx

Keywords:
2-Aminoacetophenone
Wine
Untypical aging off-flavor
UTA
HPTLC
Fluorescence detection

ABSTRACT

2-Aminoacetophenone (AAP) is closely correlated with the appearance of the sensory phenomenon of UTA ("untypical aging off-flavor") in wine. AAP analyses are generally performed by gas chromatography and mass selective detection (GC/MS), when AAP is extracted from wines by liquid–liquid, solid–liquid or solid phase microextraction. Here we present a rapid, selective and sensitive method for the determination of AAP in wine by high-performance thin-layer chromatography with fluorescence detection (HPTLC–FLD). As internal standard, 2-amino-4-methoxyacetophenone was used. Liquid–liquid extraction with *t*-butyl methyl ether was followed by a basic cleanup of the extracts, which were applied onto HPTLC amino plates developed with methylene chloride/toluene (7 + 3, v/v) as mobile phase. Dipping the dried plate into hexane-paraffin solution enhanced fluorescence that was scanned at 366/>400 nm. Limits of detection and quantitation were determined to be 0.1 and 0.3 $\mu g L^{-1}$ wine, respectively, while only AAP concentrations >0.5 $\mu g L^{-1}$ result in UTA. Recoveries were near 100% for model, white, rosé and red wines. Thus, the HPTLC–FLD method enables the analysis of AAP in wines clearly below the odor thresholds and represents a rapid and convenient screening alternative to existing GC/MS methods.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

2-Aminoacetophenone (AAP) was discovered as the key component positively correlated to the appearance of "untypical aging off-flavor" (UTA) in wines [1], which is described with sensory characteristics such as floor polish, wet wool, naphthalene note, acacia blossom, or hybrid tone [2,3]. For the analysis of AAP in wine, several gas chromatographic methods are described in literature. The difficulties of AAP quantitation in wine arises from low concentrations and from numerous volatile compounds leading to interferences during gas chromatography (GC). Rapp et al. [4] developed a method to quantify AAP in model solutions, using 2,4-dichloroaniline as internal standard. After sample extraction for 15 h with trichlorofluoromethane the solvent was carefully evaporated through a Vigreux column. Analysis by GC coupled to mass spectrometry (MS) resulted in an LOD of 0.8 $\mu g L^{-1}$. The application of a nitrogen-specific detector (NPD) improved the sensitivity to 0.02 $\mu g L^{-1}$, but two-dimensional GC (MDGC) was required for separation of AAP from other nitrogen compounds of the wine matrix [4]. Schmarr et al. [5] described a method based on solid phase extraction on LiChrolut EN with methylene chloride, followed by an alkaline washing of the extracts, when d_3-AAP was used as internal standard. After the addition of *n*-heptane as a keeper, the solvent was evaporated at room temperature. Although MDGC/MS was applied, interfering matrix effects were still observed in the second dimension, but were successfully overcome by selected ion monitoring (SIM). This method was modified by Ganß et al. [6], who quantified AAP in sparkling wines. Dollmann et al. [7] also used d_3-AAP as internal standard, but in contrast to the former studies, they adjusted the wine samples to pH 8 before extraction with n-pentane for 16 h, followed by concentration through a Vigreux column. For GC/MS analysis, columns of different polarities were applied [7], depending on interfering compounds in different wines. Nevertheless, the authors reported relative standard deviations of up to 40% in off-flavored wines, attributed to varying interfering compounds in the matrix wine [7]. Similar methods were described by Christoph et al. [8] and Hühn et al. [9], who used ethyl vanillin and naphthalene, respectively, as internal standards and extracted wine samples with trichlorofluoromethane [8] and methylene chloride [9], but reduced the extraction times to 7 h and 12 h, respectively. After the extracts were concentrated through a Vigreux column, GC in combination with mass selec-

* Corresponding author. Fax: +49 711 459 24096.
E-mail address: wolfgang.schwack@uni-hohenheim.de (W. Schwack).

http://dx.doi.org/10.1016/j.chroma.2015.12.081
0021-9673/© 2015 Elsevier B.V. All rights reserved.

Please cite this article in press as: N. Horlacher, W. Schwack, Determination of 2-Aminoacetophenone in wine by high-performance thin-layer chromatography—fluorescence detection, J. Chromatogr. A (2015), http://dx.doi.org/10.1016/j.chroma.2015.12.081

tive detection [8,9] and N-chemoluminescence detection [9] was applied. Besides liquid–liquid extraction, direct immersion [10,11], headspace [12] or ultrasound assisted headspace [11] solid-phase microextraction (SPME) were studied, when d_8-acetophenone [10], 2-nitroacetophenone [11] or d_5-AAP [12] were used as internal standards and GC/MS analyses free from interferences were reported.

General disadvantages of the reported methods include time consuming extraction and concentration steps as well as time consuming GC/MS analyses [4–12]. Therefore, the aim of the present study was to develop a rapid screening method for the determination of AAP in wine by high-performance thin-layer chromatography (HPTLC), quite different to GC. The strong native fluorescence of AAP was utilized for sensitive and selective detection not interfered by the huge number of typical wine flavor compounds. Additionally, HPTLC allows large volume applications to save concentration of extracts and to guarantee high sensitivity. The great selection of solvents for the mobile phase in normal phase HPTLC should allow the development of a selective chromatographic system to determine AAP without interferences.

2. Experimental

2.1. Reagents and materials

2-Aminoacetophenone (AAP) (98%), ethanol (≥99.8%), *t*-butyl methyl ether (TBME) (≥99.8%), hexane (≥95%), toluene (for pesticide residue analysis) and magnesium sulfate anhydrous (≥97%) were purchased from Sigma–Aldrich (Steinheim, Germany). 2-Amino-4-methoxyacetophenone (MeOAAP) (97%) was obtained from abcr (Karlsruhe, Germany), methylene chloride (≥99,9%) and paraffin oil (Ph. eur.) from Carl Roth (Karlsruhe, Germany). Sodium hydroxide pellets (≥99%) and sodium chloride (≥99,5%) were from Merck (Darmstadt, Germany). Magnesium chloride hexahydrate (pure) and L(+)-tartaric acid (pure) were purchased from AppliChem (Darmstadt, Germany). Potassium pyrosulfite was from Hefereinzucht Schlag (Aalen, Germany). ENVI-Carb was purchased from Supelco (Bellafonte, PA, USA). Enviro-Clean Bulk Chlorofiltr was from UCT (Bristol, PA, USA). Water was obtained from a Synergy ultrapure water system (Merck Millipore, Schwalbach, Germany). HPTLC silica gel 60 NH_2 plates were from Merck and were used without pre-washing.

2.2. Standard solutions

For the preparation of stock solutions, 2 mg of AAP and MeOAAP were individually dissolved in 20 mL ethanol (100 mg L^{-1}). Spiking and internal standard solutions of 0.5 mg L^{-1} AAP and 1.0 mg L^{-1} MeOAAP were achieved by diluting the stocks 1:200 and 1:100, respectively, with ethanol. Further dilution by 1:20 and 1:6, respectively, with TBME resulted in standard solutions of AAP (25 μg L^{-1}) and MeOAAP (167 μg L^{-1}).

2.3. Sample preparation

As samples, a model wine [5] and commercially available wines from a local supermarket were used. For extraction, 30 mL of wine were transferred into 50-mL Teflon FEP (fluorinated ethylene propylene) centrifuge tubes equipped with Tefzel (ethylene-tetrafluoroethylene) screw caps (Thermo Scientific, Rochester, USA) and containing 3 g of sodium chloride. After the addition of 50 μL MeOAAP internal standard solution and 3 mL TBME, extraction was performed for 1 h on an orbital shaker at 270 min^{-1}. For a clear phase separation, the samples were centrifuged at 2576 × *g* at –5 °C for 30 min. A 1.5-mL aliquot of the TBME phase was pipetted into a 12-mL screw-capped glass vial and shaken with 500 μL

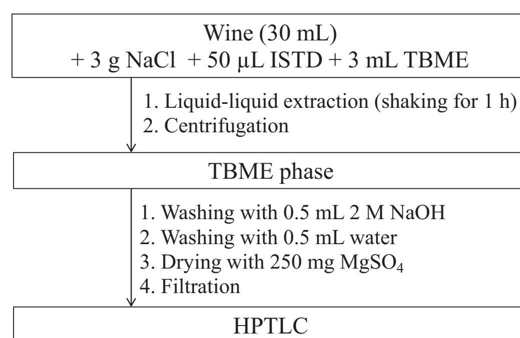

Fig. 1. Analytical procedure for the determination of AAP in wine.

of a 2 M NaOH solution for 5 min at 270 min^{-1}. The aqueous phase was removed and the procedure repeated with 500 μL of water. The TBME extract was dried over 250 mg of magnesium sulfate by shaking for 30 s at 270 min^{-1} and transferred through a 0.45-μm filter tip (Macherey-Nagel, Düren, Germany) into an HPTLC vial. The whole sample preparation procedure is sketched in Fig. 1. For recovery experiments, wines were spiked with 30, 60, or 90 μL of the AAP standard solution, resulting in AAP concentrations of 0.5, 1.0, and 1.5 μg L^{-1}, respectively.

2.4. HPTLC

Sample and standard applications were performed by an automatic TLC sampler ATS 4 (CAMAG, Muttenz, Switzerland) with the following settings: area application (6 mm × 3 mm); 22 tracks on a 20 cm × 10 cm plate, track distance 8.5 mm, 8 mm from the lower edge and 10 mm from the left side. Application volumes were 200 μL for sample extracts and 10, 20, 40, 60, 80, 120, and 140 μL for AAP standard solution, resulting in 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, and 3.5 ng/area, expressed as 0.125, 0.25, 0.5, 0.75, 1.0, 1.5 and 1.75 μg AAP L^{-1} wine for calibration. AAP standard areas were oversprayed with 20 μL of MeOAAP internal standard solution. To avoid long application times, dosage speed was set to 1200 nL s^{-1} and the nozzle temperature to 40 °C. Development was performed in the automated developing chamber ADC 2 (CAMAG) equipped with a 20 cm × 10 cm twin-trough chamber. Humidity was controlled before development to 33% relative humidity by saturated magnesium chloride solution (550 g L^{-1}) for 5 min. As mobile phase, a mixture of methylene chloride and toluene (7 + 3, v/v) was used; migration distance was 60 mm (migration time 11 min). The plate was dried inside a fume cupboard for 10 min, dipped into hexane/paraffin oil (2 + 1, v/v) (Chromatogram Immersion Device III (CAMAG), immersion speed 3 cm/s, immersion time 3 s) for fluorescence enhancement, and then dried for 2 min under a stream of cold air. Plate images were captured with the TLC Visualizer (CAMAG) under 366 nm. With a scanning speed of 20 mm/s and a slit dimension of 4 mm × 0.45 mm, the plate was scanned in fluorescence mode at 366/>400 nm by the TLC Scanner 4 (CAMAG); scan range: 20–50 mm; quick scan range: 25–45 mm, track for quick scan: highest AAP standard. Some red wines required an additional cut off at 500 nm. Therefore, a 500 nm Techspec shortpass filter (Edmund optics, Barrington, NJ, USA) was mounted onto the K400 filter (CAMAG) for fluorescence measurement at >400 and <500 nm. For the internal standard mode calibration, the applied standards were expressed as μg AAP L^{-1} wine, and the peak heights were evaluated. HPTLC instruments were controlled and calculations were performed by winCATS Software 1.4.6.2002 (CAMAG).

Please cite this article in press as: N. Horlacher, W. Schwack, Determination of 2-Aminoacetophenone in wine by high-performance thin-layer chromatography—fluorescence detection, J. Chromatogr. A (2015), http://dx.doi.org/10.1016/j.chroma.2015.12.081

Download English Version:

https://daneshyari.com/en/article/7610307

Download Persian Version:

https://daneshyari.com/article/7610307

Daneshyari.com