PTR-MS in enology: Advances in analytics and data analysis

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Abstract

The present communication deals with the improvement of proton transfer reaction mass spectrometry (PTR-MS) wine headspace analyses. In contrast to previous PTR-MS investigations of wine, where wine headspace was ionized by protonated ethanol clusters, the headspace was diluted by a factor of 1:40 with N_2 and ionized by H_3O^+ ions. This method is better suited for routine applications than the previously reported method since it is simpler, faster, and the mass spectra obtained are less complex. A test wine was mixed with ethanol and with water to yield ethanol contents ranging from 10 to 15% (v/v) and these mixtures were analyzed to assess whether any quantitative differences in the composition of volatiles were detectable. The data showed no impact of the ethanol content on the wine headspace composition. The new method was applied to eight different wine samples produced from two different grape varieties: *Pinot Noir* and *Cabernet Sauvignon*. Each variety was grown in two different locations in South Tyrol (Northern Italy) and harvested at two different dates. Quantitative (but not qualitative) differences in PTR-MS spectra between the two wine varieties were observed. Using principal component analysis of selected *m/z* signals differentiation between *Pinot Noir* and *Cabernet Sauvignon* samples was achievable.

1. Introduction

Proton transfer reaction mass spectrometry (PTR-MS) is an analytical technique for the detection and quantification of volatile organic compounds (VOCs) in air. PTR-MS offers a wide range of features suitable for food quality assessment. For instance, no sample preparation is needed, which is both time-saving and avoids adulteration of samples before analysis. PTR-MS is a highly sensitive (low pptv range) analytical method based on chemical ionization (CI) by non-dissociative proton transfer reactions, resulting predominantly in signals assignable to quasi-molecular ions $[MH]^+$. The primary ion usually used for CI is H_3O^+ and since most VOCs exhibit proton affinities higher than H_2O, H_3O^+ ions are suitable for the protonation of a large variety of VOCs including aldehydes, ketones, esters,

carboxylic acids, etc. [1,2]. PTR-MS enables the continuous on-line detection of changes in the composition and quantity of volatiles in food samples, e.g., during food processing [3], fruit storage [4], and food consumption [5] via direct headspace VOC analysis. The performance of PTR-MS is comparable to electron ionization mass spectrometry (EI-MS) in terms of linearity, resolution, and detection limit [6]. However, PTR-MS data are one-dimensional and therefore the differentiation of isobaric and isomeric compounds is not possible under standard operating conditions [7].

More than 800 volatile compounds have been detected in wine; among them are several isomeric and isobaric compounds [8]. The qualitative and quantitative composition of wine volatiles depends on various factors like grape variety, climatic factors, and viticulture practices, as well as storage and enological techniques [8]. The bouquet of wine changes continuously from the moment of harvesting to the moment of consumption. Typical alcohol levels of red wines range from 10 to 15% (v/v) and exceed other aroma compounds by a factor of 10^3–10^6 [8,9]. The solubility of volatiles generally increases in the presence of

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ethanol with a corresponding decrease in headspace concentrations of these compounds [8–10]. In contrast, an experiment investigating the changes of 18 volatiles in the headspaces of aqueous versus ethanol solutions showed a delay in the decrease of most volatiles during dynamic headspace measurements in the presence of ethanol [10].

Besides its influence on solubility and headspace concentration of volatile compounds, ethanol has an impact on the PTR-MS ionization processes. In the presence of high levels of ethanol H_3O^+ primary ions predominantly react to form protonated ethanol monomers ($C_2H_5OHH^+$, *m/z* 47), dimers [$C_2H_5OHH^+(C_2H_5OH)$, *m/z* 93], trimers [$C_2H_5OHH^+(C_2H_5OH)_2$, *m/z* 139], H_2O-adducts [$C_2H_5OHH^+(H_2O)$, *m/z* 65] as well as fragment ions due to H_2O elimination $C_2H_5^+$, *m/z* 29 and mixed $C_2H_5^+$ ethanol clusters [$C_2H_5^+(C_2H_5OH)$, *m/z* 75; $C_2H_5^+(C_2H_5OH)_2$, *m/z* 121] [11] which will subsequently react with other organic trace volatiles. For 21 simple esters these fragmentation patterns were studied in detail [12].

Absolute and relative abundances of the various ethanol product ions depend on the ethanol concentration. Conclusively, headspace analyses of otherwise identical samples containing different concentrations of ethanol might yield divergent mass spectra. Recently, a 10-fold dilution of wine headspace into an ethanol-saturated N_2 flow was proposed to generate a constantly high level of ethanol in the analyte gas [11]. The resulting mass spectral fingerprints of wine headspace are independent of the ethanol content in the wine sample. The associated change in the ionization chemistry has, however, some negative implications. H_3O^+ ions are replaced by $C_2H_5OHH^+(C_2H_5OH)_{n=1,2}$ ions as primary reagent ions. $C_2H_5OHH^+(C_2H_5OH)_{n=1,2}$ ions are less reactive CI reagents, analytes with low polarity or low proton affinity may no longer be subjected to ionization. Furthermore, $C_2H_5OHH^+(C_2H_5OH)_{n=1,2}$ ions predominantly react in ligand switching reactions resulting in the formation of mixed protonated VOC–ethanol clusters. The qualitative interpretation of the resulting mass spectra is very complicated.

Here, an alternative method for PTR-MS headspace analyses of wine samples based on a 40-fold dilution of wine headspace into pure N_2 is presented. Employing this new method, wine samples from different varieties, growing site altitudes, and harvesting dates were differentiated without prior adjustment of their original ethanol content.

2. Experimental

2.1. PTR-MS parameters

Method development and quantification studies were performed on a high sensitivity proton transfer reaction mass spectrometer (hs-PTR-MS, Ionicon Analytik, Innsbruck, Austria). Technical specifications of the PTR-MS instrument and technique are described in literature [1,14]. The sample inlet system as described in [11] was applied, with the only modification that the sample headspace was diluted 40-fold into pure N_2 (Alphagaz 1, 99.999%, Air Liquide Italia, Milano). Prior to PTR-MS analysis, 15 ml aliquots of wine were transferred to

40 ml glass vials capped with PTFE (Polytetrafluoroethylene, Teflon) septa, and the glass vials were kept in a water bath at 25 °C for 10 min. A constant flow of 2 ml min^{-1} N_2 was introduced through a gas-tight syringe into the sample headspace and exported through a second syringe. The sample headspace was then diluted into an 80 ml min^{-1} flow of N_2. Thus, the ethanol (*m/z* 47) to primary ion (H_3O^+) ratio in the drift tube was constantly kept below 10%. The diluted sample gas was transferred through a heated (60 °C) 40 cm PTFE tube (inner diameter 1.59 mm) to provide a uniform mixture. This PTFE tube was connected to the Silcosteel® inlet of the PTR-MS instrument. The PTR-MS was set up with an inlet temperature of 80 °C and a sample gas flow to the PTR-MS instrument of 70 ml min^{-1}; the 12 ml min^{-1} overflow was discarded. The instrument was operated at an E/N (electric field strength to molecule number density in the reaction chamber) of 140 Td (1 Td = 10^{-17} cm^2 V molecule^{-1}) and in "mass scan" mode in the range of *m/z* 20–200, with a dwell time of 0.2 s per *m/z* (36 s per cycle). The H_3O^+ abundance was 1.2×10^7 counts per second (cps). Decay of the primary ion signal during the analyses did not exceed 5%. The abundances of O_2^+ ions (*m/z* 32) and of H_3O^+ (H_2O) clusters (*m/z* 37) were <1%.

Every sample was measured in triplicate over 10 scan cycles. During the first 5 cycles (minutes 1–3) equilibration of the system was achieved. Cycles 6–10 (minutes 3–6) were used for data evaluation. Residual compounds were removed from the PTFE tubes and Silcosteel® inlet by introducing 10 ml min^{-1} N_2 into a vial containing purified water (analytical/laboratory grade, Millipore) instead of wine. After this procedure all signal intensities were back to baseline levels.

2.2. Preparation of wines with different ethanol concentrations

To investigate whether different ethanol contents in the range of 10–15% (v/v) had any significant effect on the quantitative results of PTR-MS wine headspace analyses, a test wine was adjusted to different alcohol concentrations (10, 11, 12, 13, 14, and 15%, v/v) by adding either water (analytical/laboratory grade, Millipore) or ethanol (Ethanol absolute extra pure, Merck, Darmstadt, Germany) (Table 1). The wine used for dilution was

Table 1
Dilution series of a *Pinot Noir* test wine for assessing the influence of different ethanol contents on PTR-MS analyses of wine headspace

% vol[a]	H$_2$O [ml]	Ethanol [ml]	*Pinot Noir* undil. [ml]	dilution factor[c]
10	28.6	–	71.4	1.40
11	21.4	–	78.6	1.27
12	14.3	–	85.7	1.17
13	7.1	–	92.9	1.08
14[b]	–	–	–	1.00
15	–	1.2	98.8	1.01

[a] Final ethanol content.
[b] For this test concentration the undiluted *Pinot Noir* (*Pinot Noir* undil.) containing 13.97 % (v/v) Ethanol was used.
[c] Calculated as 100/*Pinot Noir* undil. [ml].