

A sophisticated setup for rapid, sensitive and selective food and flavor analysis

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Abstract

A novel Proton Transfer Reaction – Mass Spectrometry (PTR-MS) setup for rapid, sensitive and selective food and flavor analysis is introduced and proof-of-concept measurements are presented. The setup enables rapid and sensitive analysis because of the utilization of ion transmission improving technologies, namely an ion funnel and a hexapole ion guide between the PTR drift tube and the mass spectrometer and the interfacing with an autosampler. Furthermore, the setup is highly selective because of the implementation of an advanced fastGC inlet system. Using a certified gas standard, the performance of the PTR-MS instrument is evaluated and compared to conventional devices. Finally, the combination of all instrumental components is tested in real-life conditions by analyzing nine different red wines.

Introduction

For decades Gas Chromatography – Mass Spectrometry (GC-MS) has been the gold standard for sensitive and selective analysis in food and flavor science. However, soon after its introduction in the 1990s PTR-MS has proven its potential in this field and has rapidly become an established method for real-time monitoring [1]. Early PTR-MS instruments were equipped with quadrupole mass filters and lacked selectivity because of unit mass resolution. They provided real-time quantification capability only for monitoring of selected compounds, as acquiring full mass spectra with quadrupole MS can only be done in mass scanning mode and thus is time-consuming. These drawbacks have been overcome by the introduction of high resolution Time-Of-Flight (TOF) mass spectrometers in the late 2000s [1], [2]. Nowadays PTR-TOFMS instruments can be considered as state-of-the-art, because of their ample advantages over quadrupole based PTR-MS devices and development work of various commercial manufacturers and universities is mainly focused on the further improvement of selectivity and sensitivity.

The importance of high selectivity in food and flavor science is obvious, as often a large number of compounds needs to be analyzed in complex matrices (e.g. coffee [3], wine [4], etc.), with many of them being isobars or even isomers. Whereas high mass resolution of TOF mass spectrometers enables the separation of isobaric compounds, isomers cannot be distinguished regardless of the resolution and require additional means of selectivity improving measures. Early attempts of coupling GC to PTR-MS were successful in considerably improving selectivity (separation of isomers, unambiguous compound identification, etc.) but disabled one of the most important advantages of PTR-MS, namely the real-time capability [5]. Much more rapid methods, which have subsequently evolved, include switching of reagent ions and changing of the reduced electric field strength in the PTR drift tube (E/N) [6], but have not met the gold standard GC in terms of selectivity so far. Eventually, a presumably ideal compromise between separation power and response time has been published in 2014: a rapid GC system consisting of a multi-capillary-column coupled to PTR-TOFMS [7].

The main advantage of improved sensitivity is not only a better Limit-of-Detection (LoD), but increased measurement speed and better quality of data. If one assumes a typical sensitivity of 25 cps/ppbv of first generation PTR-TOFMS instruments [2], the relative statistical error for measuring a compound at 1 ppbv for 1 s is 20% (square root of count rate divided by count rate). Exactly the same concentration measured for 1 s with an improved instrument with 1000 cps/ppbv would lead to an error of only 3%. Even if the measurement time would be reduced by one order of magnitude to 100 ms, the error would still be only 10%. Thus, sensitivity is of utmost importance for time-critical applications, such as flavor analysis in mouth- and nosespace or rapid aroma releasing processes. Furthermore, sample throughput can be considerably increased if the measurement time per sample can be reduced.

Here we present a sophisticated setup combining a novel high-sensitivity PTR-TOFMS instrument with an advanced fastGC inlet system, which allows for switching between direct injection and fastGC mode, and an autosampler.

Experimental

The working principle of PTR-TOFMS has been described in detail e.g. in the book by Ellis and Mayhew [8]. In short, H_3O^+ , NO^+ , O_2^+ [9] and Kr^+ [10], respectively, reagent ions are generated in a hollow cathode ion source and injected into a drift tube, where chemical ionization of the analytes takes place. The reagent and product ions are then separated according to their m/z in a TOF analyzer and detected with a microchannel plate detector.

The transfer region between the drift tube and the TOF mass spectrometer is what distinguishes the novel instrument (called "PTR-TOF 6000 X2") from conventional designs. Recently, it has been found that this area, traditionally consisting of a series of electrostatic lenses, is the cause for a high amount of ion losses and thus is the main limiting factor for the overall sensitivity. As a countermeasure we developed an ion funnel for being installed at the end of the drift tube. Ion funnels have been invented in the late 1990s [11] and consist of a series of lenses with successively smaller apertures to which an alternating voltage is applied. This setup effectively focuses ions to the exit aperture and has first been applied to PTR-TOFMS by Barber *et al.* [12], where the ion funnel constituted about 50% of the drift tube and thus formed a major part of the reaction region. We, however, developed a compact and modular funnel design, which i) is primarily for focusing the ions and with only about 1/3 of the drift tube length, not forming an integral part of the reaction region and ii) can easily be installed in existing PTR-TOFMS instruments by replacing the drift tube exit lens with the ion funnel.

In 2014 we introduced a PTR-TOFMS instrument equipped with a quadrupole ion guide instead of a conventional transfer lens system [13]. Now we considerably improved this design by developing a hexapole instead of a quadrupole ion guide, as hexapoles are known to have a better transmission and to be more beneficial for focusing ions of a broad m/z range. Besides improving the transmission, i.e. the sensitivity of the instrument, multipole ion guides additionally cause cooling of the ions and thus improve injection conditions into the TOF mass spectrometer, which results in an increased mass resolution. In the results section we present the effects this combination of ion funnel and hexapole ion guide, which is displayed in the schematic view in Figure 1, has on the instrument's sensitivity by analyzing a certified gas standard (TO-14A aromatics mix).

For selectivity improvement we essentially revised the GC design from [7] to an efficient fastGC setup. The present setup consists of an electronically switchable pressure

controlled multiport valve, which enables switching between direct injection and fastGC mode. In direct injection mode the air from the sampling line is split to two lines, with one line leading directly to the PTR drift tube and the other feeding a sample loop. That is, while the instrument is measuring in real-time, as it is common for PTR-MS, the sample loop is continuously flushed with sample air. By switching to the fastGC mode, the content of the sample loop is injected into a 10 m nonpolar MXT-1 column, which can be heated to 400°C with a heating rate of up to 1200°C/min. After being separated according to their retention times the compounds are injected into the drift tube. Typical spectral runs take between 30 to 150 s depending on the temperature profile. This means that by switching between direct injection and fastGC mode the advantages of PTR-MS and GC can be combined, namely real-time analysis and the highest level of selectivity.

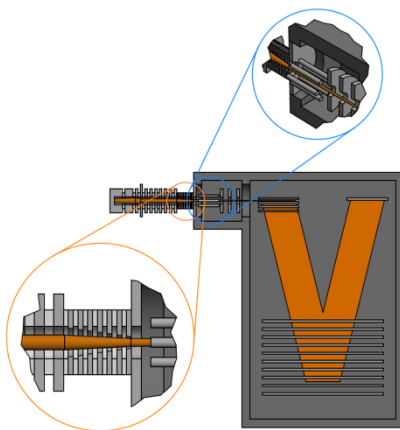


Figure 1: Schematic view of the novel PTR-TOF 6000 X2 equipped with an ion funnel (insert bottom left) and hexapole ion guide (insert top right).

The "sophisticated setup for rapid, sensitive and selective food and flavor analysis", as referred to in the title of this contribution, is completed by a commercial autosampler (PAL RSI, CTC Analytics AG, CH) for which we developed a dedicated interface to connect it to the PTR-TOFMS instrument. This interface consists of a heated cell, which is constantly flushed with N₂ at a controlled flow rate. Via a septum the content of the autosampler syringe is injected into the cell. Finally, the mixture of headspace and N₂ is introduced into the PTR-TOFMS instrument via a common inlet line and excess air is ejected via an overflow port. This design is necessary so that the static headspace of sample vials can be analyzed, because the PTR-TOFMS instrument requires a continuous sample gas flow.

As a proof-of-concept test of the performance of the novel setup we investigated nine different red wines purchased at a local supermarket.

Results and discussion

In order to evaluate the sensitivity improvement of installing an ion funnel and a hexapole ion guide we analyzed a certified TO-14A aromatics mix with three different PTR-TOFMS instruments. In the left diagram of Figure 2 the results are shown. The PTR-TOF 1000 is a conventional instrument with a system of electrostatic lenses in the transfer region between the drift tube and the TOF mass spectrometer. The PTR-TOF 1000 *ultra* has been upgraded by installing a modular ion funnel, as described in the experimental

section. Finally, the PTR-TOF 6000 X2 is equipped with a hexapole ion guide in addition to the ion funnel and thus combines both transmission improving technologies. However, it should be noted that the latter instrument has a TOF with a longer flight-path, compared to the PTR-TOF 1000, which improves the mass resolution, but somewhat lowers the sensitivity, because a lower pulse rate has to be used. All instruments show increasing sensitivities with increasing m/z , which is a well-known effect observed in TOF analyzers. For the conventional PTR-TOF 1000 the resulting sensitivity is in the range of 60 – 130 cps/ppbv. After installing the modular ion funnel the sensitivity is boosted by nearly one order of magnitude to 600 – 1000 cps/ppbv for the PTR-TOF 1000 *ultra*. Eventually, the combination of further improved transmission due to the hexapole ion guide and a lower pulse frequency leads to a boost by another factor of 2 for the PTR-TOF 6000 X2, with values between 1200 – 1800 cps/ppbv.

In the right diagram of Figure 2 the minimum integration times for an arbitrary compound with a low concentration of 100 pptv in order to get a relative statistical error of 5 and 10% (compare introduction), respectively, have been calculated using the measured sensitivities. Assuming one wants to quantify 100 pptv with 5% error e.g. in exhaled nospacespace air, the importance of high sensitivity gets immediately obvious. The 30 s of the PTR-TOF 1000 well exceed the duration of a breath cycle and disqualify the instrument for the given task. With the PTR-TOF 6000 X2, however, 100 pptv can be detected with 5% error within about 2 s and if an error of 10% is acceptable, the breath cycles can be monitored with a high time resolution of 600 ms.

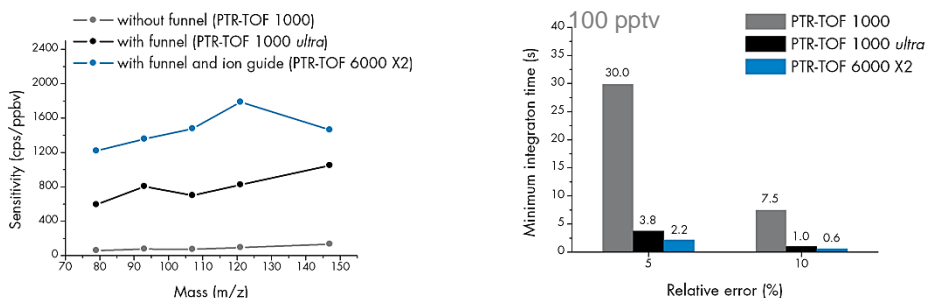


Figure 2: Comparison of measured sensitivities for different instrument types (left) and the calculated minimum integration times to reach a relative error of 5 and 10%, respectively (right).

For the LoDs, calculated via the maximum sensitivities and by using the 3σ method, we determined 70, 10 and 10 pptv for 1 s and 10 pptv, 750 ppqv and 550 ppqv for 1 min integration time for the PTR-TOF 1000, *ultra* and 6000 X2, respectively. The mass resolutions (full width at half maximum) at m/z 181 were 1700 $m/\Delta m$ for the PTR-TOF 1000 (*ultra*) and 6000 $m/\Delta m$ for the PTR-TOF 6000 X2 (data not shown).

Figure 3 shows the instrumental response during the autosampler injection of wine headspace while switching between direct injection and fastGC mode. The various lines in this diagram represent the ion yields for different m/z versus time. *Note:* As data evaluation of the wine study is still ongoing and this contribution should only serve as a proof-of-concept, here we do not attempt to identify compounds or go into detail about the different wines. As soon as the autosampler injects the wine headspace into the interface an immediate response can be seen for all m/z . In conventional PTR-TOFMS these signal intensities would be attributed to distinct compounds matching the exact m/z .

However, switching to fastGC mode unveils that for nearly all m/z more than one ion is contributing to the total ion yield, which can be due to the presence of isomers, non-resolved isobars or fragment ions.

The advantage of high selectivity gets even more obvious in Figure 4, where the nine different wines are compared. At m/z 131.11 two ions can be separated in fastGC mode (left diagram). Importantly, these two ions, which are detected as one sum signal in direct injection mode, have considerably different ratios. That is, even if the ion yields at a particular m/z are similar in intensity for several wines in direct injection mode, it is still possible that the compounds contributing to this ion yield are present in completely different concentrations. This can also nicely be seen in the right diagram of Figure 4, where even three ions can be seen at m/z 117.09. For some wines the abundance of all three ions is comparable in intensity, whereas for other wines over 50% originates from the ion at 38 s retention time.

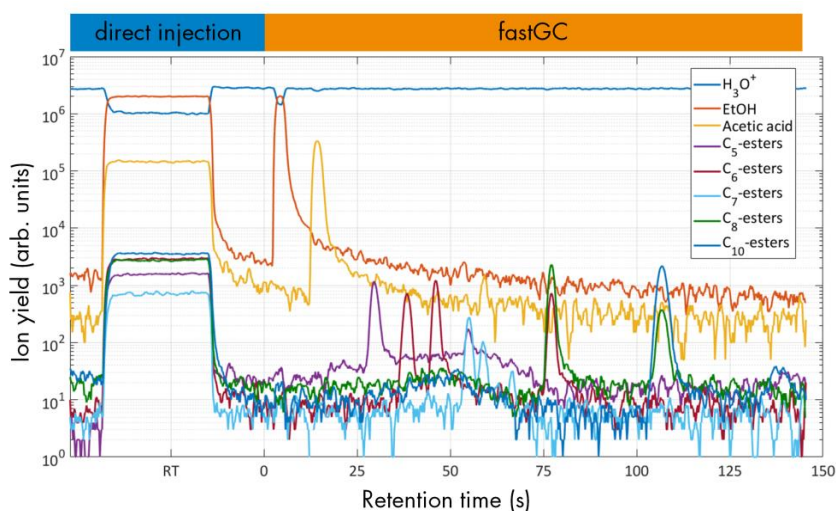


Figure 3: Exemplary ion yields during the autosampler injection of wine headspace in direct injection and fastGC mode.

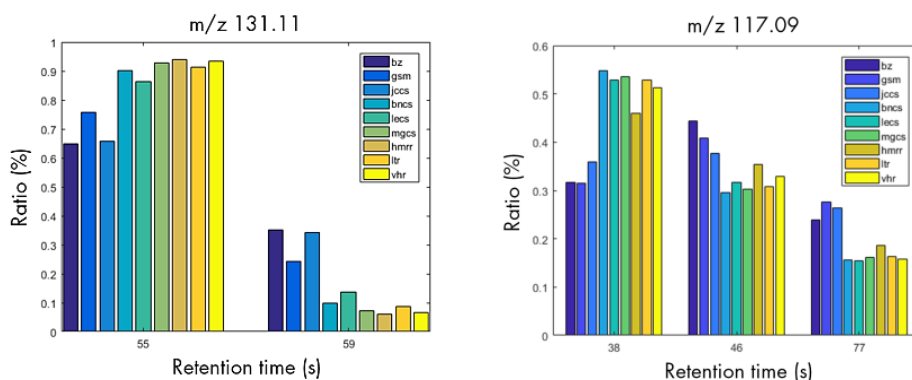


Figure 4: Relative intensity distributions for different ions sharing the same exact m/z : 131.11 (left) and 117.09 (right); the abbreviations stand for the different wines.

We conclude that the combination of an ion funnel and hexapole ion guide in a high resolution PTR-TOFMS instrument with additionally a fastGC and autosampler inlet system is a powerful setup for rapid, sensitive and selective food and flavor analysis, which produces considerably more high quality data at a higher sample-throughput than established methods. As a next step following this proof-of-concept we will perform statistical analysis on the acquired data in order to distinguish between different brands/vintages of wine.

Acknowledgement

FP has received funding through the IMPACT ITN which is supported by the European Union's H2020 Programme under Grant Agreement Number 674911. Furthermore, we gratefully acknowledge financial support from the Land Tirol via an "Innovationsförderung" and the FFG via ASAP projects.

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