

Monoterpenes separation by coupling Proton Transfer Reaction Time of Flight Mass Spectrometry with fastGC

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Abstract

Proton Transfer Reaction Mass Spectrometry (PTR-MS) is a well-established technique for real-time VOCs (Volatile Organic Compounds) analysis. Although, it is extremely sensitive (with sensitivities of up to 4500 cps/ppbv, limits of the detection < 1 pptv and the response times of approximately 100 ms) the selectivity of PTR-MS is still somewhat limited, as isomers cannot be separated. Recently, selectivity-enhancing measures, such as manipulation of drift tube parameters (reduced electric field strength) and using primary ions other than H_3O^+ , such as NO^+ and O_2^+ have been introduced. However, monoterpenes, which belong to the most important plant VOCs, still cannot be distinguished so that more traditional technologies, such as gas chromatography mass spectrometry (GC-MS), have to be utilized. GC-MS is very time consuming (up to 1 h) and cannot be used for real-time analysis.

Here we introduce a sensitive, near real-time method for plant monoterpene research: PTR-MS coupled with fastGC. We successfully separated and identified six of the most abundant

monoterpenes in plant studies (α - and β -pinenes, limonene, 3-carene, camphene, and myrcene) in less than 80 s, using both standards and conifer branch enclosures (Norway spruce, Scots pine and Black pine). Five monoterpenes usually present in Norway spruce samples with a high abundance were separated even when the compound concentrations were diluted to 20 ppbv. Thus, fastGC-PTR-ToF-MS was shown to be an adequate one-instrument solution for plant monoterpene research.

Key words

PTR-MS, fastGC, monoterpenes, VOC, plant VOCs, pinene

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Introduction

Monoterpenes are a group of compounds emitted in high quantities by numerous plant species, especially conifers. The most abundant plant monoterpenes are α - and β -pinene, limonene, 3-carene, camphene, and myrcenes [1–3]. Monoterpenes have many ecologically related functions: 1) plant injury protection (conifers resin), 2) pollinator attraction, 3) fruit and seed dispersal (zoochory), and 4) they are very important food aroma compounds. Moreover, monoterpenes are emitted into the atmosphere in amounts that affect our climate globally via aerosol and cloud formation [4].

Plant and atmosphere monoterpene research requires sensitive analytical techniques among which the most important are: 1) Proton Transfer Reaction Time of Flight Mass Spectrometry (PTR-ToF-MS) and 2) Thermal Desorption Gas Chromatography Mass Spectrometry (TD GC-MS) [1, 5–7]. Other techniques such as Selective Ion Flow Tube Mass Spectrometry (SIFT-MS), and other, GC based, techniques are also used, but usually for samples with higher monoterpene concentrations [1, 3, 8, 9].

PTR-MS is a real-time technology with potential for plant monoterpene emission measurements at below 1 s time resolution, with sensitivities of up to 4500 cps/ppbv and limits of detection <1 pptv [10]. This way any rapid change in VOC emission can be monitored in real-time. In the last two decades the sensitivity and performance of PTR-MS has improved [5, 7, 11, 12]. However, like all chemical ionization technologies, PTR-MS cannot separate isomers in monoterpene blends, which are usually present in nature. A step forward to a better qualitative analytical performance of PTR-MS has been the usage of different E/N value settings (where the reduced electric field strength E/N is the ratio between the electric field, E , and the number gas density, N , in the drift tube; it is directly related to the collision energy applied to the ion-molecules) resulting in different product ion branching ratios of the compounds [5, 13]. Moreover, recent development in PTR-MS made available usage of other primary ions such as NO^+ and O_2^+ , which further increases the analytical power of this real-time technique [14, 15]. However, to our knowledge no separation of monoterpenes by PTR-MS is yet possible for analysis of different monoterpene concentrations in a rich natural mix.

TD GC-MS, however, is a powerful analytical technique that can separate all monoterpene isomers, but with the disadvantage of a time resolution up to 1 h (for plant monoterpene emission analysis, including the sampling time). So, the state-of-the-art approach for monoterpene experiments is to use PTR-MS in parallel with a GC based system (usually TD trapping at sampling stage, or direct loop sampling with GC-FID) [6, 16]. This requires the use of two instruments (with corresponding need for expertise in the operation and maintenance) and two types of data analysis. A one-instrument solution (coupling GC with PTR-MS) would be ideal, but in early development a huge time resolution cost remained because of lengthy analysis of GC [17]. Development of fastGC (fast Gas Chromatography) coupled with PTR-MS promises much faster monoterpene separation. In general, typical fastGC differs from conventional GC as follows: 1) short, thin-film capillary column, 2) capability of fast temperature ramp (>1 °C/s), 3) fast injection system, 4) fast and sensitive detector, 5) automated sampling, and 6) time resolution <5 min [18]. Thus, fastGC is ideal for connecting in series with PTR-MS for the lowest time resolution price. This would allow near to real-time VOC monitoring needed in plant sciences, where rapidly induced and case-

specific VOC emission patterns often arise due to herbivory, changes in metabolism and, exposure to oxidative and other stresses.

The aim of this work was to develop a near to real-time separation method of plant common monoterpenes using a fastGC coupled with PTR-ToF-MS.

Materials and methods

Standards: For this experiment the following monoterpene standards were used: (+)- α -pinene ($\geq 98.5\%$, Fluka), (+)- β -pinene ($\geq 98.5\%$, Fluka), (+)-3-carene ($\geq 98.5\%$, Fluka), camphene (95%, Sigma Aldrich), myrcene ($\geq 90\%$, Sigma Aldrich) and R-(+)-limonene (97%, Sigma Aldrich).

PTFE bags containing trace gas levels of individual monoterpenes and a bag containing a mixture of the standards were prepared to determine the retention times. For each bag the following procedure was adopted: a) approximately 1 μL of each monoterpene standard was placed in a 10 mL glass vial, closed with a PTFE septum cap and left for couple of minutes to equilibrate; b) 200-400 μL of the vial's headspace were injected in 5 L PTFE bags, previously filled with zero air (hydrocarbon free air). For the mixture bag, 200 μL of the headspace of each standard were put in a single 5 L PTFE bag, previously filled with zero air.

Sample preparation: We harvested a branch of Scots pine (*Pinus sylvestris*), black pine (*Pinus nigra*) and Norway spruce (*Picea abies*) in the suburban area of Innsbruck (Austria). A small part of the branch (Norway spruce and Scots pine), or a pair of needles (black pine), were enclosed in a leaf cuvette entirely made of PTFE (except a quartz glass window). In order to produce the desired final monoterpene concentration, sharp scissors cuts were made on a couple of needles in order to generate high monoterpene emissions, followed by zero air flow tuning (diluting the sample).

FastGC-PTR-ToF-MS: All standards and samples were analysed using a PTR-TOF 8000 (IONICON Analytik, Austria) coupled with a fastGC add-on (boxed, Version 1.04, Hardware revision 04, IONICON Analytik, Austria). The fastGC setup and mode of operation is explained elsewhere [19]. In the present study, the following instrumental parameters were used: PTR drift tube: E/N 140 Td (1 Td = 10^{-17} V/cm); fastGC: a) carrier gas flow of 3 mL/min

under standard conditions; b), injection time 4 s, temperature ramp consisting of: 6 s at 28 °C, heat to 80 °C at 49 °C/min, 40 s at 80 °C, heat to 180 °C at 150 °C/min. Each run was stopped after 80 s, after which the system was ready for the next injection in less than 10 s. We used nitrogen as a carrier and make-up gas. For each run the total monoterpene concentrations were estimated via online PTR-ToF-MS measurement (*direct injection mode*), and then the instrument was switched to *fastGC mode* to generate a chromatogram. Some details on the fastGC system may be found elsewhere [19]; however, we have used a system with faster heating and cooling rates (30 °C/s both).

PTR-ToF-MS calibration: The instrument was calibrated using a Gas Calibration Unit (GCU-a, IONICON Analytik, Austria), for dynamic dilution of a calibration gas containing 16 VOCs in the 1 ppmv range (custom made standard, Praxair NV, Belgium), including α -pinene (1.02 ppmv). The limit of detection (LoD) of the fastGC system was determined using the 3σ method on 20 runs of the background [20]. To evaluate the instrument's sensitivity for each monoterpene, dilutions of the (+)- α -pinene standard and consecutive fastGC runs were carried out. Sensitivities were evaluated using both m/z 137.1325 and the sum of m/z 137.1325 and m/z 81.0699, since the latter had already been identified as a fragment ion of protonated monoterpenes [6, 13].

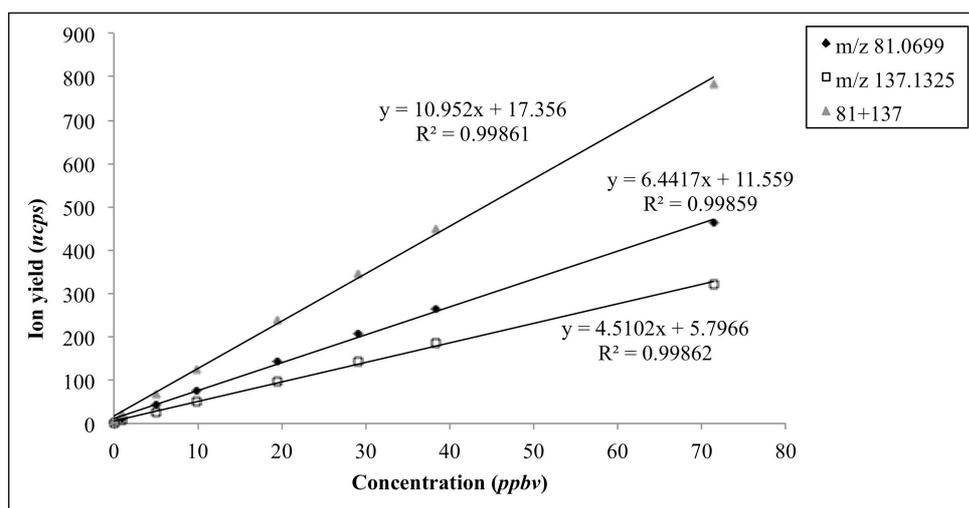


Fig. 1 PTR-ToF-MS calibration graph (direct injection mode). Values obtained using Gas Calibration Unit, with a calibration gas standards containing 1.02 ppmv of α -pinene

Data analysis: PTRMS Viewer 3.0 (IONICON Analytik, Austria) was used to process the data for the following primary ions: H_3O^+ (m/z 21.0226), $\text{H}_2\text{O}.\text{H}_3\text{O}^+$ (m/z 37.0290), and $(\text{H}_2\text{O})_2.\text{H}_3\text{O}^+$ (m/z 55.0395); and product ions: monoterpenes (m/z 81.0699 and m/z 137.1325). The product ion signals were normalised to one million primary ions (the sum of H_3O^+ , $\text{H}_2\text{O}.\text{H}_3\text{O}^+$, and $(\text{H}_2\text{O})_2.\text{H}_3\text{O}^+$). FastGCpeakCalc script was used to evaluate the peak area of each monoterpene in the fastGC chromatogram [21]. The script takes input parameters (file names, peaks starts and ends) and calculates the peak areas for each extracted ion (m/z 81.0699 and m/z 137.1325), and saves them in a report file. The signal to noise ratio (S/N) is calculated as uncorrected normalised peak area (ncps) divided by the average background, that integrated from the same peak parameters.

To avoid confusion between *direct injection mode* and *fastGC mode* we present PTR-ToF-MS online measurements in normalised counts per second (ncps), and fastGC data in normalised counts (nc) or ppbv.

Results

The calibration curve for the PTR-TOF 8000 is shown Fig. 1. The calculated LoD (α -pinene) of fastGC was 1.2 ppbv (8.5 nc) using m/z 137.1325, and 2 ppbv (54 nc) using both m/z 81.0699 and m/z 137.1325. The calculated LoD of the other compounds are given in Table 1; however, the real values may differ as fragmentation patterns may be different than in α -pinene. The sensitivities obtained for m/z 81.0699 + m/z 137.1325 and m/z 137.1325 were 26.94 nc/ppbv and 6.90 nc/ppbv, respectively (Fig. 2). Note that the LoD values in *fastGC mode* measurements are significantly higher compared to expected values for PTR-MS (<1 pptv). This is mainly caused by three factors: 1) the required make up gas effectively dilutes the sample eluting from the fastGC, 2) the sub-pptv levels of PTR-MS detection are usually achieved with much longer integration times (couple of minutes), and 3) the fastGC signals are integrated in the chromatogram where each compound has different peak width.

Table 1. LoD for all of the monoterpenes used in the present study (fastGC mode). Note that calibration had been carried out using α -pinene; therefore volume mixing ratios (ppbv) may differ for other monoterpenes as fragmentation patterns may be different. Legend: nc – normalised counts, m81 – m/z 81.0699, m137 – m/z 137.1325. * Concentration [ng/L] = Concentration [ppbv] x 136 [g/mol] / 24.45 [L]

LoD	α -Pinene	Camphene	β -Pinene	Myrcene	3-Carene	R-Limonene
m81+m137 [nc]	54.5	38.0	27.3	27.3	27.8	18.5
m137 [nc]	8.5	8.5	11.4	9.0	10.4	9.5
m81+m137 [ppbv]*	2.0	1.4	1.0	1.0	1.0	0.7
m137 [ppbv]*	1.2	1.2	1.7	1.3	1.5	1.4

A mixture containing six monoterpenes was analysed using FastGC-PTR-ToF-MS and each single monoterpene may be identified in less than 80 seconds (Fig. 3a). The separation was complete except in the case of α -pinene/camphene. Comparing the retention times of 5 repetitions for each compound yields an average relative standard deviation in retention time of 0.15 s +/- 0.07 s (0.45% +/- 0.25%), thus a repeatability (inherent precision) < 1%.

Six monoterpenes were identified in the chromatogram of Norway spruce (Fig. 3b). An additional unidentified chromatogram signal could be observed at a retention time (RT) of 77 s. We tentatively attribute this to a monoterpene, which was not present in our standard mixture. Furthermore, a significant chromatogram signal for m/z 81.0699 was observed at a RT of 24 s. We excluded this signal to derive from an additional monoterpene, since we did not observe any related chromatographic answer on m/z 137.1325 [6].

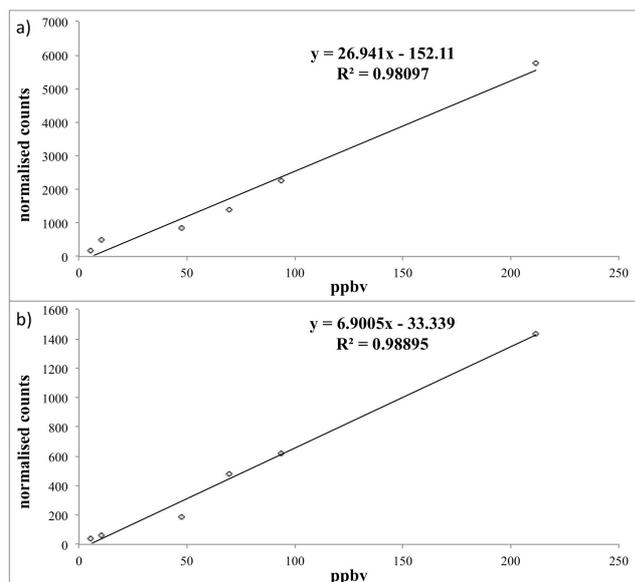


Fig. 2 FastGC-PTR-ToF-MS calibration graph (fastGC mode). Sensitivity obtained using dilutions of (+)- α -pinene standard (212, 92, 69, 47, 11 and 5 ppbv). a) Analysis performed using sum of m/z 81.0699 and m/z 137.1325 ions, b) analysis performed using only m/z 137.1325 ion chromatograms

High amounts of α - and β -pinene were observed in the case of both pine species (Figure 3C and 3D). Here again an overlap of highly abundant α -pinene and traces of camphene may be observed. Furthermore after the limonene peak, in both cases, one or two more unidentified monoterpenes were seen (RT 72 and 77 s).

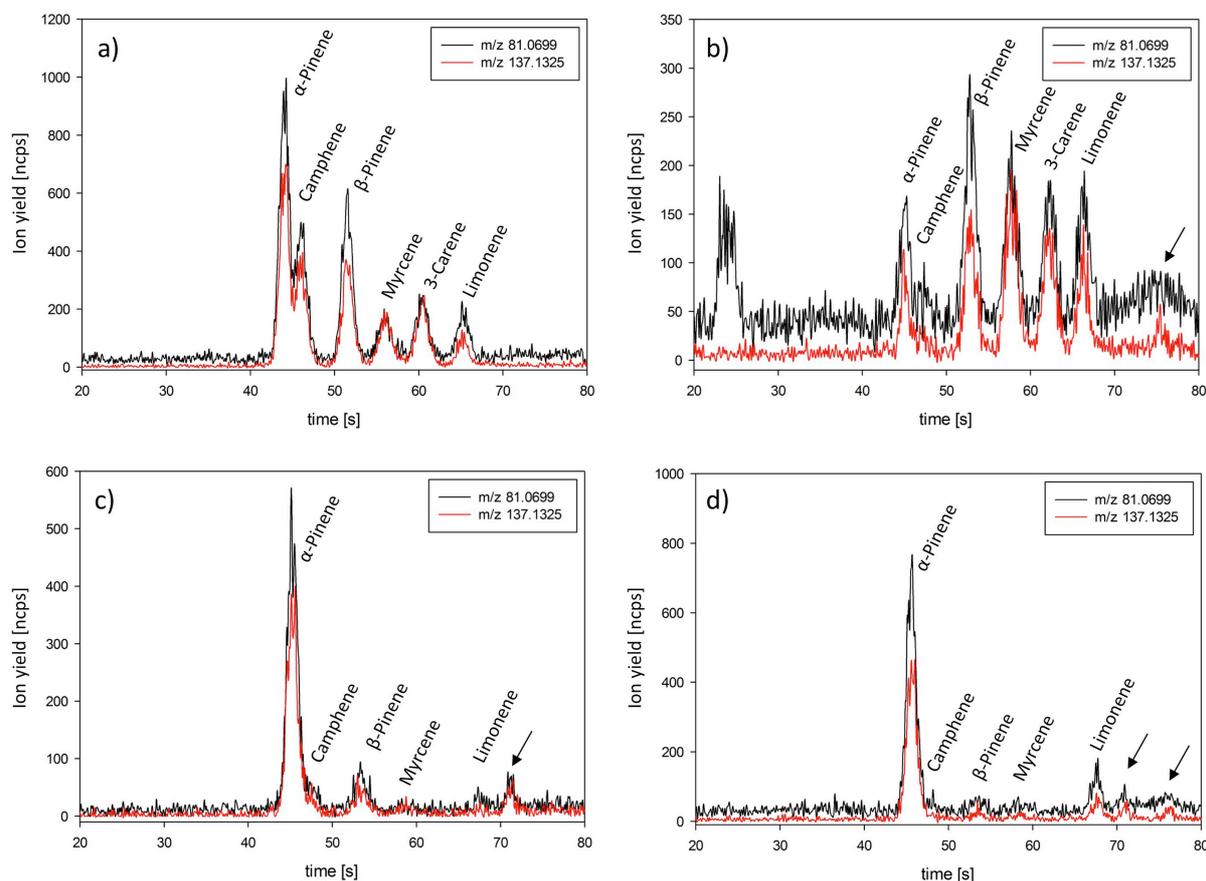


Fig. 3 FastGC-PTR-ToF-MS chromatograms of: a) Monoterpene standards, 750 ppbv (see material and methods section), b) Norway spruce samples, 180 ppbv (note the unidentified monoterpene $S/N = 3.35$), c) Scots pine samples, 200 ppbv (S/N : α -pinene 26.29, camphene 2.58, β -pinene 3.31, myrcene 1.62, limonene 1.25, with an unidentified monoterpene $S/N = 4.00$) and, d) Black pine samples, 220 ppbv (S/N : α -pinene 30.62, camphene 2.19, β -pinene 1.94, myrcene 1.83, limonene 4.46, with unidentified monoterpenes 3.45 and 2.67 respectively). Arrows indicate unidentified monoterpenes

As an additional test for low monoterpene concentrations, spruce samples with a total monoterpene concentration of 20 ppbv (evaluated using online PTR-ToF-MS measurements), were analysed using the fastGC system. Unlike pines, which are abundant in only one or two monoterpenes, spruces emit a high concentration of six monoterpenes. As can be observed in Fig. 4, the peak areas of most monoterpenes are above the LoD.

However, better signal to noise ratios are obtained when using only m/z 137.1325, instead of the sum of m/z 81.0699 and m/z 137.1325. Also one more peak (α -pinene) was found above LoD when analysed using m/z 137.1325 ion chromatogram (Fig. 4b).

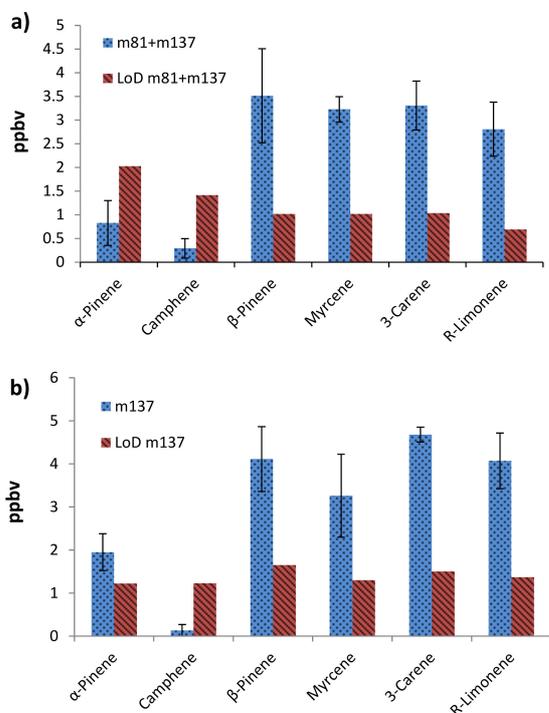


Fig. 4 Peak areas of Norway spruce chromatogram obtained in fastGC mode using total monoterpene concentrations of 20 ppbv. Error bars represent standard deviation on two replicates. a) Analysis performed using sum of m/z 81.0699 and m/z 137.1325 ions, S/N: α -pinene 0.82, camphene 0.78, β -pinene 1.55, myrcene 1.55, 3-carene 1.61, limonene 2.04, b) Analysis performed using only m/z 137.1325 ion chromatogram, S/N: α -pinene 1.13, camphene 0.57, β -pinene 1.49, myrcene 1.74, 3-carene 1.80, limonene 1.67 (one additional monoterpene above the limit of the detection).

Discussion

Monoterpenes measured by PTR-MS produce two major fragments: m/z 81 and m/z 137 [13]. In order to measure the monoterpene signal by fastGC-PTR-ToF-MS we either used the sum of m/z 81 and m/z 137 or just m/z 137. Our results suggest that a lower LoD is achieved for α -pinene if just m/z 137 is used (Table 1). Furthermore, lower values of the sensitivity follow this pattern, suggesting that only m/z 137 should be used, when analysing samples with a low concentration of monoterpenes. Moreover, m/z 81 may be related to other compounds and/or compound fragment ions, for example green leaf volatiles such as (*E*)-2-

hexenal and (*Z*)-3-hexenal, which may be found in complex samples such as plant VOCs [22, 23]. On the other hand, using just *m/z* 137 could lead to some inaccuracy as the ion branching ratios of each monoterpene may differ for different monoterpenes when analysed under different *E/N* conditions [13].

The fastGC method was optimised to obtain monoterpene separation in less than 2 minutes (Fig. 3a). However, working with complex samples such as conifers, which usually contain VOCs with a high boiling point, occasionally required heating the column to higher temperatures (180 °C) for the last 10-20 seconds of the fastGC run.

Experiments on Norway spruce samples showed the full potential of this method for the identification and separation of all six of the most abundant monoterpenes. In addition, the analysis of the spruce sample (Fig. 3b), showed the identification capability whether a chromatographic signal is generated by a monoterpene compound (see the chromatographic signal at a RT of 24 s), thus illustrating the potential of the system for the compound identification and separation, using multi ion chromatograms and potentially the deconvolution approach [24].

In the pine chromatograms, although α -pinene is the most abundant compound, we observed the presence of camphene, β -pinene, myrcene and limonene. However, no 3-carene signal was observed. This might be explained either by low 3-carene emitting tree specimen [1]; or by species specific seasonality in monoterpenes blend [3].

The capabilities of this technique were verified with the analysis of low concentration spruce monoterpenes (20 ppbv of total monoterpenes, 4-6 ppbv per individual monoterpene). This shows that fastGC-PTR-ToF-MS may be used in real plant VOCs experiments and atmospheric chemistry research as the ultimate online plus near real-time approach. However, further upgrades of the system are possible and will decrease the LoD and improve the sensitivity and separation capabilities.

The method is not only limited to monoterpene research since it can also be used for other applications (e.g. separation of sesquiterpenes and green leaf volatiles), and can be inexpensively optimised by developing new fastGC methods (temperature ramp, injection

time, flows, total run time, etc.), swapping the carrier gas (usage of He instead N₂) and changing the column type and length.

Conclusions

Plant monoterpenes are a compound group, which has numerous isomers carrying diverse ecological and biological functions. Until now, the method of monoterpene analysis was to monitor the emission by a real-time instrument (PTR-MS) and analyse the individual monoterpenes by a GC system (TD-GC-MS).

For the first time, we achieved a near real-time monoterpene separation and identification by coupling fastGC and PTR-ToF-MS. We successfully separated and identified six monoterpenes using both monoterpene standards and plant material (branches) in less than 80 seconds (up to 10 s required between sampling). We measured low limit of the detection (1.2 ppbv) and high sensitivity (6.9 nc/ppbv) of the system. We successfully separated and identified the five spruce monoterpenes at a total monoterpene concentration of 20 ppbv.

Thus, the combination of online measurement (by PTR-MS) and measurement in fastGC mode (by fastGC-PTR-MS), can be applied as the all-in-one-instrument solution of monoterpene research, resulting in real-time emission measurements and more than 6 chromatograms per hour.

Conflict of interest

The measurements were conducted in the laboratory of IONICON Analytik, the manufacturer of the PTR-TOF 8000 and the fastGC. PS, JH, and ML are employed by IONICON Analytik. Other authors declare that they have no conflict of interest.

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