

PROTON TRANSFER REACTION MASS SPECTROMETRY APPROACH TO MONITOR MONOTERPENES EMISSION FROM ALEPPO PINE (*PINUS HALEPENSIS*) WOODS GROWING IN DIFFERENT LOCATIONS

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SYNOPSIS. The aim of this preliminary study was to investigate the possibility of detection and quantification of various monoterpenes emitted from wood by using Proton Transfer Reaction Mass Spectrometry; a novel procedure based on the collision-induced dissociation has been investigated. The energy of collision has been varying in a range of 80 Td to 180 Td with a step of 20 Td. Samples of Aleppo pine (*Pinus halepensis*) wood growing in France and Algeria have been measured and compared. The quantity of monoterpenes emitted from Algerian wood was much higher than from French. The most significant masses detected in both cases were m81 and m137. However, collision-induced dissociation patterns were slightly different, what suggests different composition of monoterpenes in woods from France and Algeria.

KEY WORDS: PTR-MS, wood provenience, Aleppo pine, monoterpenes

INTRODUCTION

Proton Transfer Reaction Mass Spectrometry (PTR-MS) is sensitive and real-time technique for detection and identification of volatile organic compounds (VOC). It plays a major role in analysis of environmental and atmospheric sciences. Trees (plants in general), emit many types of chemical compounds, such as isoprene, hexanal, monoterpenes, oxygenated monoterpenes and sesquiterpenes (TANI et AL. 2003). These chemicals (especially terpenes) play a fundamental role

in the defence chemistry of plants (MICHELOZZI et AL. 2004). In general, monoterpenes can be seen as a combination of two isoprene entities C_5H_8 and may be saturated, unsaturated, cyclic or acyclic. Their different isomers may vary only by as much as the position of a double bond (STEEGHS et AL. 2007).

The VOC of conifers belong mostly to the class of monoterpenoids (HILLIS 1962). Since the relative proportion of monoterpenes in mature tissues is under strong genetic control (only slightly affected by environmental parameters), monoterpenes play important role in taxonomic studies of coniferous species and are useful in forest genetics as biochemical markers (GALLIS and PANETSOS 1997, LLUSIA and PEÑUELAS 2000, MICHELOZZI et AL. 2004); geographical variations of *Pinus pinaster* monoterpene composition has been reported by TOGNETTI et AL. (2000) for example. Similar works dedicated to *Pinus nigra*, *Pinus halepensis* and *Pinus ponderosa* has been published by SMITH et AL. (1969), BOJOVIC et AL. (2005), and TAHAR et AL. (2007) respectively.

Several analytical methods can be utilized for detection and quantization of monoterpenes emitted from wood. The most commonly used today are gas chromatography (GC) and mass spectrometry (MS). Each of these techniques possesses advantages and limitations; but none can provide full characterisation alone. GC can separate volatile compounds with a great resolution, but it cannot doubtlessly identify them. MS can't readily separate mixed VOC. It can provide however detailed structural information on the molecules detected; lately used for identification. In consequence a combined gas chromatograph-mass spectrometry (GC-MS) technique has been developed. It merges great separation ability of GC and high sensitivity of MS. Unfortunately GC-MS, as other techniques, possesses important limitations; some isomeric compounds cannot be distinguished by MS and determination of positional substitutions on aromatic rings is often difficult (SETTLE 1997).

Recently developed proton-transfer reaction mass spectrometry (PTR-MS) is another alternative (to MS, GC or GC-MS) analytical technique capable to analyse VOC emitted from wood into ambient air. It uses a soft ionization method based on proton transfer from H_3O^+ ions to all compounds with higher proton affinity than water. Common constituents of air such as N_2 , O_2 , Ar, CO_2 etc. have lower proton affinities than H_2O and therefore do not interfere with the measurement. Most of the monoterpenes common in the wood have however, higher proton affinities than H_2O and thus can be monitored and quantified with detection limit down to 10 parts per trillion in volume (pptv).

The significant advantage of mass spectrometry is its high sensitivity. Beside that the measurement is relatively fast and does not need particularly excessive sample preparation such as pre-treatment and/or pre-concentration. The limitation of PTR-MS is the restricted amount of information about the composition of VOCs and rather difficult quantification of the mixtures of VOCs.

A collision-induced dissociation (CID) can also be applied in PTR-MS scanning procedure as an alternative to the standard measurements. CID is a mechanism of fragmentation of the molecular ions in the gas phase. It is possible to control the kinetic energy of ions in the drift tube of mass spectrometer by adjusting the electrical field and the gas pressure in the drift tube. The measure of such kinetic

energy is E/N . It is computed as a ratio of drift tube electric field and the gas number density and is expressed in Townsend ($1 \text{ Td} = 10^{-17} \text{ V}\cdot\text{cm}^2$).

STEEGHS et AL. (2007) measured ten monoterpenes and stated a possibility of its identification basing on the CID pattern. The ratio between different fragments depends on the kinetic energy of the ions as they exit the drift tube. This behaviour was attributed to differences in ion internal energy at different drift tube voltages. MALEKNIA et AL. (2007) reported that the relative abundances of RH^+ and fragments for terpenes were dependent on the drift tube voltage; the optimum voltage for detection of molecular ions was different for various terpenes. Similar methodology has been developed by APREA et AL. (2007) for measuring esters.

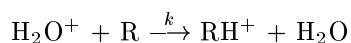
Some researchers linked the PTR-MS with measurement of monoterpenes emitted from wood or tress. RUUSKANEN et AL. (2005, 2006) applied PTR-MS for monitoring of the monoterpene emissions from the living trees continuously in the field. MÜLLER et AL. (2006) investigated biogenic volatile organic compounds (BVOC) above and within a coniferous forest. CHRISTIAN et AL. (2004) used PTR-MS with other techniques for measurement of biomass-burning emission.

The goal of this work was to investigate the possibility of detection and quantification of various monoterpenes emitted from wooden samples and to develop a novel procedure of using PTR-MS toward characterisation of chemical constituency of VOCs in wood. The work is focused on the VOCs detection from softwoods growing in various environmental conditions.

MATERIALS AND METHODS

Aleppo pine (*Pinus halepensis* Mill.) wooden blocks acquired from trees growing in France and Algeria were used as experimental samples. It was expected that due to different geographical location and different climatic conditions, the concentration and composition of monoterpenes would differ (TAHAR et AL. 2007). The wood pieces have been conditioned to 12% moisture content in the climatic chamber. Samples were cut on the circular saw just before the test to refresh the emission surface and to assure comparable emissions of monoterpenes. Each wooden piece has been cut into four blocs of dimensions 20 mm/20 mm/40 mm (thickness/width/length respectively). Blocks were stored in clean and closed containers immediately after cutting. VOC emitted from wood were measured by mass spectrometer a few hours later.

The PTR-MS spectrometer used for measurements has been developed, in collaboration with Innsbruck University, at Institute of Photonics and Nanotechnologies of Italian National Research Council. The principles of the PTR-MS technology have been described by LINDINGER et AL. (1998) and GOUW et AL. (2003). The ionization process can be summarized by the following reaction:



The protonated water H_3O^+ interacts with the measured gas R. During this interaction a proton switches from hydronium to the trace gas molecule, which

leads to a protonated (ionized) molecule RH^+ and a neutral water molecule H_2O . The probability of reaction k is usually close to or equal to the collision rate constant. This reaction only takes place when the proton affinity of the trace compound R is higher than that of water ($166.5 \text{ kcal/mol} = 7.16 \text{ eV}$). Compared to electron impact ionization, where electrons are shot at neutrals at about 70 eV , the energy involved in the reaction of proton transfer is very low. Since the excess energy of the reaction is low, fragmentation of molecule R occurs to a very limited degree; resulting in only one (or two) characteristic ions per each VOC. As a result, the mass spectra are much easier interpretable.

The PTR-MS apparatus consists of three parts:

- the ion source, where ions are produced by a hollow cathode discharge using water vapour as the molecular source of ions
- the drift tube; where proton transfer reactions to the trace constituents in the air occur
- the ion detector; which provides sensitive detection of mass specified ion scans of the molecules of interest.

The schematic of the instrument is shown in Figure 1 and more technical details can be found in the references (<http://www.ptrms.com/>).

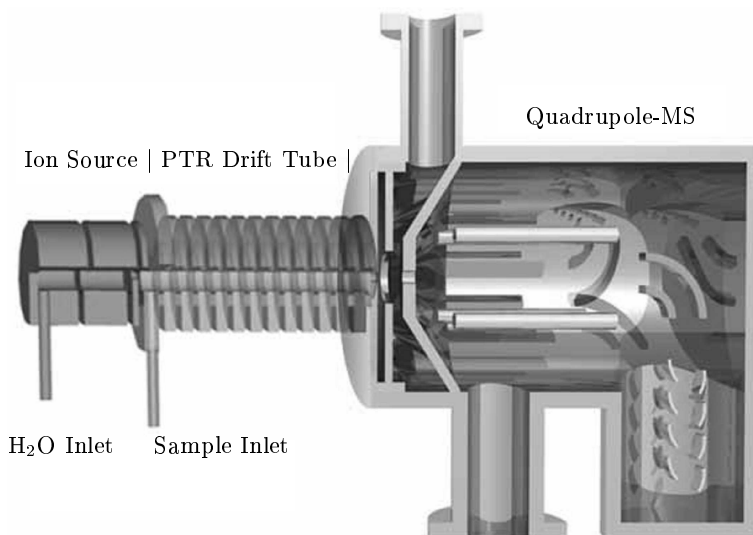


Fig. 1. Schematic of the proton transfer reaction mass spectrometer
Source: www.ptrms.com

The collision energy (E/N) in the PTR-MS spectrometer was changing during the test and it was set-up at levels of 80 Td to 180 Td with a step of 20 Td . The proper level of energy was obtained by precise manipulation of the pressure and the electric field in the reaction chamber (drift tube). The mass spectra have been collected and afterward analysed by computing the CID patterns on the self developed software.

RESULTS AND DISCUSSION

Standard PTR-MS procedure

A typical distribution of VOC masses measured from Aleppo pine originated from both Algeria and France are presented in Figure 2. The most significant concentrations of molecules detected by PTR-MS were noticed for masses 81 and 137, which can be assigned to monoterpenes emitted from wood (TANI et AL. 2003). Masses 33, 45 and 59, even if detected in significantly large amounts, have not been considered for monoterpenes analysis since these are assigned to methanol, acetaldehyde and a mixture of acetone and propane respectively. Some significant differences in mass concentrations between wood originated from Algeria and France can be noticed. It suggests different constitution of VOCs in wood from each provenance. Evidently, an amount of monoterpenes emitted from the Algerian wood was higher. It can be explained by the natural accumulation of VOCs in wood living in Africa due to water and growth stresses resulting from warm and dry Algerian climate (SCHILLER and GRUNWALD 1987, LLUSIA and PEÑUELAS 2000). On the other hand, it was impossible to estimate detailed monoterpenes composition (quality and quantity) on the base of the results obtained.

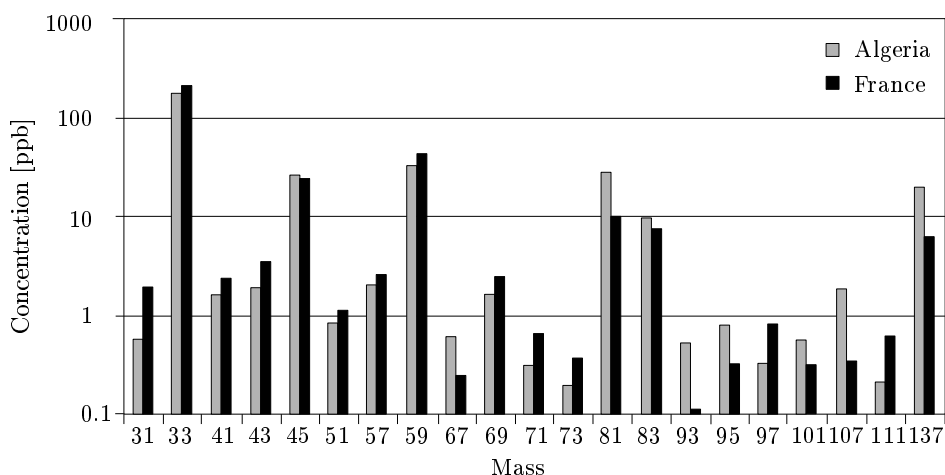


Fig. 2. Concentration of masses detected in the VOC emitted from *Pinus halepensis* originated from France and Algeria

The CID approach

Additional set of experiments has been carried out with the Aleppo pine samples in order to generate CID patterns. Untreated experimental results (for Algerian wood) are presented in Figure 3; only variations of masses m81 and m137 are shown on the graph in order to simplify interpretation. Concentration of VOC emitted from the wood (marked on the graph as a cycle wood (E/N)) have been

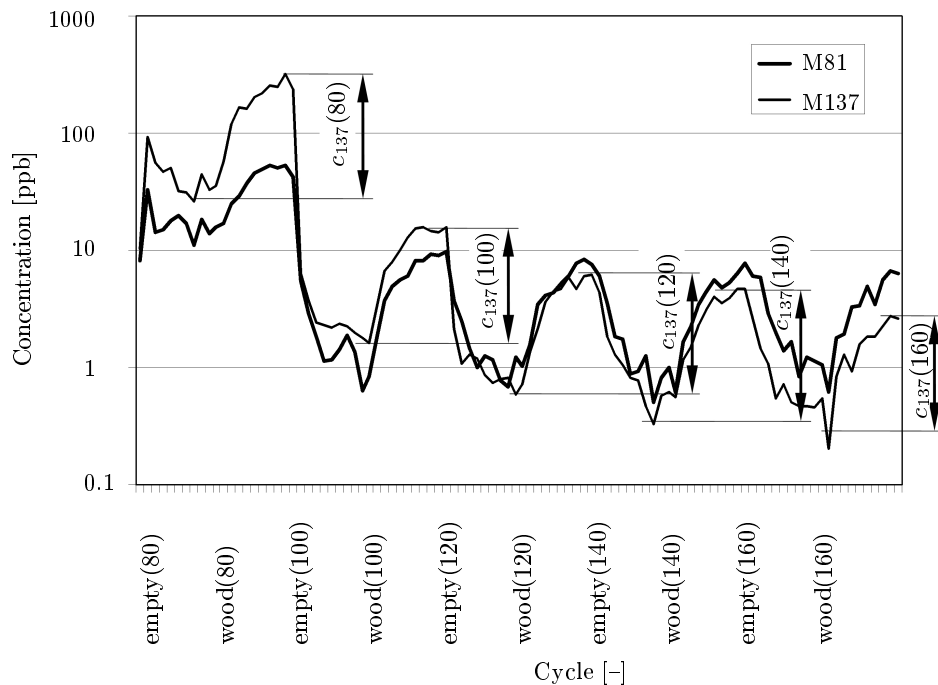


Fig. 3. Variations of the masses m81 and m137 concentration in the VOCs emitted by mediterranean pine wood in relation to the E/N energy

Note: empty (80) is a measurement moment when an empty container has been connected to the spectrometer and measured with E/N = 80 Td, wood (80) is a measurement moment when measurement of wood at the E/N = 80 Td has started.

measured with varying E/N energies. The measurement of the ambient air (empty container) has been performed as a reference (marked on the graph as cycle empty (T/N)). Each time after changing container some few cycles have been needed to stabilize the concentration level. The VOC quantities have been computed as a difference between concentration of molecules emitted from wooden sample and ambient air. It is illustrated on the Figure 3 as arrows of the length $c_{137}(T/N)$; where $c_{137}(80)$ corresponds to concentrations measured with E/N energies of 80 Td, $c_{137}(100)$ corresponds to 100 Td and so on. As can be seen there is a strong effect of the E/N energy on the effectiveness of ionization. The amount of the m137 molecules was significantly higher than m81 when the lowest energy (80 Td) has been set in the reaction chamber. The tendency was opposite with increase of the T/N. Different trends of the curves related to masses m137 and m81, as seen on the Figure 3, have been also evident. It must be mentioned that the changes of the detected molecule numbers are not related to the changes of the VOC concentration (as it is constant during the test), but rather to the effectiveness of ionization. Supplementary analyses have been performed in order to investigate this phenomenon in more detail.

Figure 4 presents the CID fragmentation patterns computed on the basis of PTR-MS examination of Algerian and French woods. The mass ratio curves

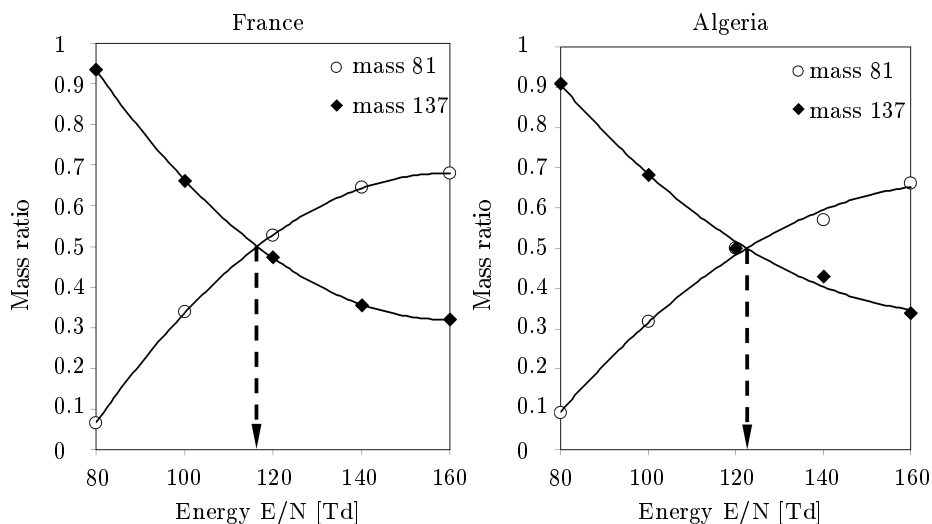


Fig. 4. Fragmentation patterns for the masses m81 and m137 of VOC emitted from French (left) and Algerian (right) woods in a function of E/N-value

of both woods have rather similar outline, but these are evidently differing in the cross-section point. While the intercept in case of wood originated from France was for $E/N \sim 115$ Td, the corresponding intercept for Algerian wood was at E/N of ~ 125 Td. It is apparent evidence that even if both samples emitted similar VOC, some differences in the monoterpene composition exists. Unfortunately, it is impossible (at the moment) to identify the origin of differences and exact meaning of the shift. However, recent works are focused on improving the data mining and developing novel PTR-MS methodologies for such assessments.

CONCLUSIONS

The work presented here demonstrates an applicability of PTR-MS technique for measuring VOC emission from wood originating from different sites. The most significant concentrations of molecules detected by PTR-MS were noticed for masses m81 and m137, which can be assigned to monoterpenes emitted from wood. Some differences in mass concentrations between wood originated from Algeria and France were observed; it suggests different constitution of VOC in wood from each provenance. The new methodology of VOC measurement with PTR-MS by varying E/N energy and CID pattern analysis is very promising development and it has a great potential for applications related to measurement of monoterpenes in wood.

Acknowledgement

Part of this work has been carried out with a financial support of Fondazione Cassa di Risparmio di Trento e Rovereto (project BiQUEEN). The support was highly acknowledged.

REFERENCES

- APREA E., BIASIOLI F., MÄRK T.D., GASPERI F. (2007): PTR-MS study of esters in water and water/ethanol solutions: Fragmentation patterns and partition coefficients. *Int. J. Mass Spectr.* 262, 1-2: 114-121.
- BOJOVIC S., JURC M., DRAZIC D., PAVLOVIC P., MITROVIC M., DJURDEVIC L., DODD R., AFZAL-RAFIH Z., BARBERO M. (2005): Origin identification of *Pinus nigra* populations in southwestern Europe using terpene composition variations. *Trees* 19: 531-538.
- CHRISTIAN T.J., KLEISS B., YOKELSON R.J., HOLZINGER R., CRUTZEN P.J., HAO W.M., SHIRAI T., BLAKE D.R. (2004): Comprehensive laboratory measurements of biomass-burning emissions: 2. First intercomparison of open path FTIR PTR-MS and GC-MS/FID/ECD. *J. Geophys. Res.* 109 (D2): 2311.
- GALLIS A.T., PANETSOS K.P. (1997): Use of cortical terpenes to discriminate *Pinus brutia* (Ten.) *Pinus halepensis* (Mill.) and their hybrids. *Silv. Genet.* 46, 2-3: 82-88.
- GOUW J.A., WARNEKE C., KARL T., EERDEKENS G., VAN DER VEEN C., FALL R. (2003): Sensitivity and specificity of atmospheric trace gas detection by proton-transfer-reaction mass spectrometry. *Int. J. Mass Spectr.* 223-224: 365-382.
- HILLIS W.E. (1962) Wood extractives and their significance to the pulp and paper industries. Academy Press, New York.
- LINDINGER W., HANSEL A., JORDAN A. (1998): Proton-Transfer-Reaction Mass Spectrometry (PTR-MS): On-line monitoring of volatile organic compounds at pptv levels. *Chem. Soc. Rev.* 27: 347-354.
- LLUSIA J., PEÑUELAS J. (2000): Seasonal patterns of terpene content and emission from seven Mediterranean woody species in field conditions. *Am. J. Bot.* 87(1): 133-140.
- MALEKNIA S.D., BELL T.L., ADAMS M.A. (2007): PTR-MS analysis of reference and plant-emitted volatile organic compounds. *Int. J. Mass Spectr.* 262(3): 203-210.
- MICHELOZZI M., TOGNETTI R., MAGGINO F., RADICATI M. (2004): Seasonal variations in monoterpene profiles and ecophysiological traits in Mediterranean pine species of group "halepensis". *Forest* 1(1): 37-50.
- MÜLLER K., HAFERKORN S., GRAMBER W., WISTHALER A., HANSEL A., KREUZWIESER J., COJOCARIU C., RENNENBERG H., HERRMANN H. (2006): Biogenic carbonyl compounds within and above coniferous forest in Germany. *Atmosph. Environ.* 40: 81-91.
- RUUSKANEN T.M., KOLARI P., BÄCK J., KULMALA M., RINNE J., HAKOLA H., TAIPALE R., RAIVONEN M., ALTIMIR N., HARI P. (2005): On-line field measurements of monoterpene emissions from Scots pine by proton-transfer-reaction mass spectrometry. *Boreal Environ. Res.* 10: 553-567.
- RUUSKANEN T., RINNE J., TAIPALE R., KOLARI P., BÄCK J., HAKOLA H., HELLÉN H., KERONEN P., ALTIMIR N., HARI P., KULMALA M. (2006): Measurements of biogenic VOCs in a boreal forest ecosystem. *Proc. 2nd Conf. on Proton Transfer*

- Reaction Mass Spectrometry and its Applications. January 29 – February 03, 2005, Obergurgl, Austria. Eds. A. Hansel, T. Märk. Institut für Ionenphysik der Leopold-Franzens-Universität, Innsbruck: 108-111.
- SCHILLER G., GRUNWALD C. (1987): Resin monoterpenes in range-wide provenance trails of *Pinus halepensis* Mill in Israel. *Silv. Genet.* 36(3-4): 109-114.
- SETTLE F.A. (1997): Handbook of instrumental techniques for analytical chemistry. Prentice Hall PTR, Upper Saddle River.
- SMITH R.H., PELOQUIN R.L., PASSOFF P.C. (1969): Local and regional variation in the monoterpenes of Ponderosa pine wood oleoresin. U.S.D.A. For. Serv. Res. Pap. PSW-56: 1-10.
- STEEGHS M.M.L., CRESPO E., HARREN F.J.M. (2007): Collision induced dissociation study of 10 monoterpenes for identification in trace gas measurements using the newly developed proton-transfer reaction ion trap mass spectrometer. *Int. J. Mass Spectr.* 263: 204-212.
- TAHAR D., TAYEB B., CHAABANE C. (2007): Essential oil composition of *Pinus halepensis* Mill. from three different regions of Algeria. *J. Ess. Oil Res.* 19(1): 40-43.
- TANI A., HAYWARD S., HEWITT C.N. (2003): Measurement of monoterpenes and related compounds by proton transfer reaction-mass spectrometry (PTR-MS). *Int. J. Mass Spectr.* 223-224: 561-578.
- TOGNETTI R., MICHELOZZI M., LAUTERI M., BRUGNOLI E., GIANNINI R. (2000): Geographic variation in growth, carbon isotope discrimination, and monoterpene composition in *Pinus pinaster* Ait. provenances. *Can. J. For. Res.* 30(11): 1682-1690. <http://www.ptrms.com/>, cited at 31 October 2008.

Received in July 2009

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