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Development of a simple gradient LC–IR interface for the detection of terpenoids from the α-pinene–ozone reaction

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Dedicated to Professor Dr. Dieter Klockow on the occasion of his 67th birthday.

Abstract

The development of a simple interface between liquid chromatography and infrared spectroscopy (LC–IR) using a coaxial sprayer is described for less volatile analytes. The system consists of a transfer capillary, in which the analytes are transported from the separation column of the gradient-LC to the outlet of the sprayer. This transfer capillary is coaxially surrounded at the outlet by a stainless steel sprayer capillary, which is resistively heated and flushed with nitrogen gas. The samples are sprayed in the manner that the eluent is vaporized by the heated nitrogen when exiting the capillary, while the analytes are deposited on a moving slide made of infrared transparent material (ZnSe or CaF_2). Afterwards the deposited compounds are analyzed with an infrared microscope in transmission. First results from reaction products of the gas phase reaction of α-pinene with ozone are presented.

Keywords: Interfaces, LC-IR; Infrared spectrometry; Gradient elution; Terpenoids; α-Pinene; Ozone

1. Introduction

Infrared spectroscopy (IR) has long been known to be a very useful tool for the determination of organic compounds, especially when used for the identification of specific functional groups. Since the demands for analytical methods are becoming as complex as the analytical mixtures, the coupling of spectroscopic methods with chromatographic separation is necessary.

The combination of gas chromatography and infrared spectroscopy (GC–IR) has been successfully employed for the determination of different volatile organic compounds [1,2]. Infrared spectroscopy has also shown to be an important tool to identify oxygen containing functional groups from reaction products resulting from the gas phase ozonolysis of α-pinene [3].

Some products of this reaction are found in the gas phase, while others, like carboxylic acids, are believed to undergo a so-called gas-to-particle conversion. They form aerosols which are no more applicable to gas chromatography [4]. In these cases

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a separation with liquid chromatography (LC) is necessary. Although several interfaces for the combination of liquid chromatography with infrared spectroscopy (LC–IR) have been introduced, still technical difficulties are present.

Compared to GC–IR, the main problem with LC–IR is that all reversed-phase solvents used in liquid chromatography show a distinct infrared spectrum and are also present in high concentration compared to the analyte [5]. The application of flow cells has therefore been very limited [6–10].

As an alternative to the flow cell the solvent elimination interfaces have been introduced. Different interfaces have been developed with the purpose to deposit the analyte after the LC-separation solvent free in a small spot on a substrate, on which it can be spectroscopically studied. For solvent elimination, different spray techniques were applied, including concentric flow nebulization [11–14], ultrasonic nebulization [15,16] thermospraying [17,18] and hydrodynamic focusing in a so-called spray jet assembly [19]. Additionally a monodisperse aerosol generation interface (MAGIC)-LC–FTIR interface has been used [20–22], which is a modified version of the MAGIC-LC–MS interface developed by Willoughby and Browner [23]. All of the mentioned techniques have been successfully applied in LC–IR analysis of substances of rather high molecular masses like proteins [24–26], steroids [27], polymers [14,28] and their additives [29]. For these applications commercial LC–IR-systems are available. For a good summary on LC–IR, two reviews have been published recently by Somsen et al. [30,31]

The reaction products of α-pinene ozonolysis are classified into volatile and low volatile compounds. Volatile compounds can be analyzed with gas chromatography while the low volatiles can only be separated adequately with liquid chromatography. Although these low volatile compounds can not be determined with GC — among the synthetic standards available, just pinonic acid was volatile enough to be detected — they still have rather low boiling points compared to the higher molecular compounds that have been analyzed with LC–IR before. All reports concerning LC–IR separation with a solvent elimination interface deal with higher molecular species, while reports on smaller molecules are still missing, which can be attributed to the difficulties of depositing them. Nonetheless, an approach is made here to investigate the low volatile reaction products of the α-pinene–ozone gas phase reaction by using a laboratory-built LC–IR interface based on the studies of Visser and his co-workers [19,32]. The assembly developed here is rather simple and easy to build from an instrumental point of view, is inexpensive and allows a successful application of gradient reversed-phase chromatography of these compounds.

2. Experimental

The chemicals were purchased from the following vendors: hexane (Lichrosolv grade), acetone and methanol (analytical reagent grade), acetonitrile (gradient grade), acetic acid (analytical grade), ammonium acetate (analytical grade) and water (LiChrosolv grade) from Merck (Darmstadt, Germany); *cis*-pinonic acid, *cis*-pinic acid, *trans*-norpinic acid and pinolic acid from Sigma–Aldrich (Deisenhofen, Germany).

3. LC–IR-system

The LC–IR-system used consists of three main parts: the HPLC-system, the laboratory-built interface and the IR-microscope. After separation the compounds were deposited solvent free on an IR-transparent slide. Afterwards the traces were investigated with the microscope.

3.1. HPLC-system

The HPLC system used with the LC–IR-interface was a high pressure gradient system from Knauer (Berlin, Germany). It consisted of two piston pumps (WellChrom Maxi-Star K-1000), a dynamic mixing chamber with a volume of 260 μl, a six-port injection valve (both from Knauer, Berlin, Germany) with a 10-μl injection loop, a 1 mm column (l=15 cm, Kromasil 100 C_{18}, Restek, Bad Soden, Germany) for separation and a WellChrom spectrophotometer (K-2500) with a 3-mm cell (volume= 1 μl) for UV-detection. The solvents were degassed with helium before they entered the pumps.

Initially, a 4-mm column (l=25 cm, Eurospher 100 C_{18}, Knauer, Berlin, Germany) was used. The reason for the change to the 1-mm column was the better compatibility with the interface, which showed best performance at flow-rates between 20 and 50 μl. This corresponds well to the flow-rate of 50 μl/min used for the separation with the 1 mm column. Another advantage is the higher peak concentration in micro-HPLC.

3.2. The interface

The schematic of the interface developed in this study is shown in Fig. 1.

After UV-detection, the flow from the HPLC-system is transferred by a fused-silica capillary (CS, Langerwehe, Germany) via a three-way stainless steel T piece onto the IR transparent slide, e.g. ZnSe or CaF_2, which can be positioned by an x stage motor (LM 60/SM 440, OWIS, Staufen i Br., Germany). The solvent is vaporized and the analyte is focused using heated nitrogen, which is introduced through a stainless steel tube at the third connection of the T-piece and exits through the spray capillary also made of stainless steel (Hamilton, Darmstadt, Germany). The two capillaries are mounted coaxially in the manner that the transfer capillary protrudes about 0.6 mm outside of the sprayer capillary. The heating of the nitrogen is accomplished through a resistive heating system, consisting of the spray capillary and the nitrogen tubing as the connection. The distance between the spray capillary and the slide can be adjusted by a micrometer screw on which the sprayer is mounted.

3.3. IR-microscope

For IR-detection a Perkin–Elmer Infrared Microscope with a PE 2000 spectrometer was used. All spectra were recorded with a resolution of 8 cm^{-1} and four scans co-added. The aperture was adjusted in all experiments for the detection of a spot with the size of 100 μm×100 μm. Two types of spectral data acquisition were used: recording of spectra at a preselected spot and the linescan procedure, recording spectra along a preselected straight line every 100 μm.

3.4. Sampling of the reaction products

The reaction chamber has already been described elsewhere [3]. For the sampling of the less volatile

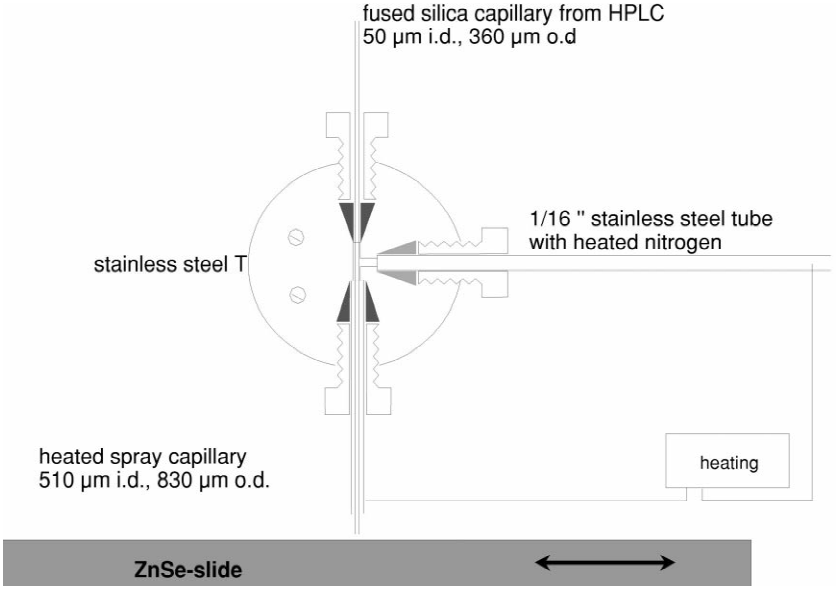

Fig. 1. LC–IR interface.

reaction products a boron silicate filter (GF6, diameter=55 mm, thickness=0.35 mm, pore size= 0.5–1.5 μm) was used (Schleicher und Schüll, Dassel, Germany) and placed in a laboratory-built holder made of Teflon at the outlet of the chamber. The gas-flow containing the products was flushed through the filter at a flow-rate of 65 l/h for 8 h. In the experiment, 6 ppm α-pinene were reacted with 4 ppm ozone. The filter was extracted after sampling with a Soxhlet extractor in 50 ml of methanol for 24 h and the resulting solution was concentrated to about 0.5 ml. For improved separation the amount of organic phase had to be reduced. Consequently, in the final preparation step 0.3 ml of H_2O were added to the solution. The final volume after evaporation of the solvents was 0.5 ml.

4. Results and discussions

4.1. LC–IR characteristics

For optimation of the analytical system, some of the basic characteristics of the interface were determined: spot size, spectra and performance during gradient separation.

Therefore, solutions of *cis*-pinonic acid were used under isocratic conditions with different compositions of the mobile phase, e.g., different aqueous phase-to-organic phase ratios and different concentrations of acetic acid. In Table 1 an overview of the tested and optimized parameters is given. The values used during gradient elution for the analysis of the filter extract are marked with bold letters.

The first experiments showed that the best results were obtained, if the flow in the inner capillary was not completely dry when it was leaving the capillary, but if the last drying step took place during the spraying process. Otherwise the inner capillary was easily blocked, by analytes crystallizing at its surface. As a consequence the length of the heated spray capillary had to be kept as short as possible, while the minimum inner diameter for the inner capillary was found to be 50 μm. The material of the inner capillary was found to be crucial. The capillaries made of stainless steel were most rapidly blocked. The fused-silica material supplied by one supplier was not temperature resistant enough, which led to easy breakage of the capillaries. Merely with the fused-silica material supplied by another supplier good results were obtained. The optimum diameter of the sprayer capillary was found to be 510 μm.

Some parameters were varied depending on the amount of water and acetic acid in the mobile phase. The nitrogen pressure and the flow-rate was decreased with increasing amount of aqueous phase and the temperature had to be increased.

In order to be able to reduce the flow in the inner

Table 1
Optimized parameter listing

Part	Parameter and variations		Optimum
Spray capillary	length	3.5, 10 cm	**3.5** cm
	inner diameter	0.31, 0.34, 0.41, 0.51, 0.60 mm	**0.51** mm
	distance from slide	0.5–3 mm	**1.2** mm
	pressure of N_2	1–7 bar	**1.5**–2 bar
	temperature at tip	0–250°C	100–**180**°C
Inner capillary	material and outer	stainless steel:	**fused silica**:
	diameter	0.21, 0.26, 0.36 mm fused silica: 0.21, 0.32, 0.36 mm	**0.36** mm
	inner diameter	25, 50, 75, 100 μm	**50** μm
	distance from slide	0.2–2 mm	**0.6** mm
	flow rate	10–500 μl/min	**20**–50 μl/min
x-stage motor	speed	25, 37.5, 50, 75 μm/s	37.5; **50** μm/s

capillary without changing the flow through the column, a split was installed between the UV-detector and the interface.

4.2. Spot size and spectra

In Fig. 2 the spot and the corresponding spectrum of *cis*-pinonic acid is displayed. The mobile phase consisted of 40% of 10 mmol acetic acid and 60% acetonitrile. A 4-mm column was used in the experiment. The flow through the column was 0.5 ml/min. A aliquot of 20 μl of a solution of 1.8 mg/ml of *cis*-pinonic acid in acetonitrile was injected. The flow was split and 1/25 was transferred into the interface, resulting in a maximum amount of 1.4 μg of analyte concentrated in one spot.

The image of the spot was recorded in the visible mode of the IR-microscope and for better contrast digitally processed. The spectrum of pinonic acid in Fig. 2 indicates that acidic dimers are formed, based on the broad absorption band between 2700 and 2500 cm^{-1}.

The spot diameter of 360 μm was characteristic for the system throughout most measurements under isocratic conditions. Larger spot sizes were obtained only if the amount of substance was high. Smaller spots were usually not obtained either. The fact that the diameter of the spot is identical with the outer diameter of the fused-silica capillary, indicates that these parameters are corresponding.

In Fig. 3 two further spots with their corresponding spectra are displayed. In these experiments the injected compound was pinic acid. A micro-HPLC-system was used for separation at a flow-rate of 50 μl/min using a 1-mm column. The concentrations of the injected solutions were 290 μg/ml for Fig. 3a and 29 μg/ml for Fig. 3b. A 10-μl aliquot was injected and no split was used, which means that the spectra corresponds to 2.9 μg and 290 ng of pinic acid, respectively. For comparison of the spot size a fused-silica capillary with an outer diameter of 180 μm was placed next to the spots. The images were recorded with a video camera [AVT MC-1307/S(F), AVT-Horn, Aalen] connected to a stereomicroscope (Stemi 2000, Zeiss, Jena, Germany).

The spectra of pinic acid also show the characteristic dimer bands. The spot diameter is again around 360 μm in Fig. 3a. The other spot (Fig. 3b) seems to be only about half the size. Upon closer inspection, however, an outer ring appears around the spot. The diameter of this ring is about 360 μm. Due to the lower amount injected, the spot was not thick enough and could probably be evaporated at the edge.

4.3. Gradient separation of organic acids

A standard mixture consisting of *cis*-pinonic acid, *cis*-pinic acid with traces of *trans*-pinic acid, *trans*-norpinic acid with traces of *cis*-norpinic acid and pinolic acid was used in order to test the performance of the LC–IR-system during gradient separation. The gradient program was used as follows: A: 32 mmol acetic acid in aqueous solution, B: acetonitrile/A (60:8, v/v); start: 97% A, after 5 min: 75% A–25% B, after 30 min: 97% B.

It should be noted that the required separation

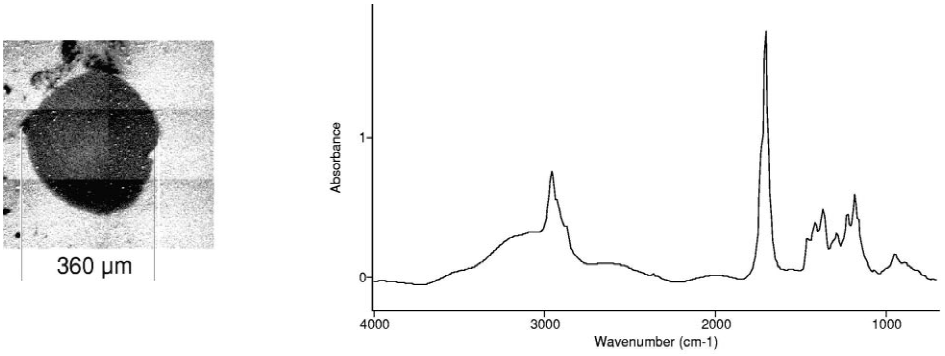

Fig. 2. Spot and corresponding IR-spectrum of *cis*-pinonic acid obtained with the LC–IR-system.

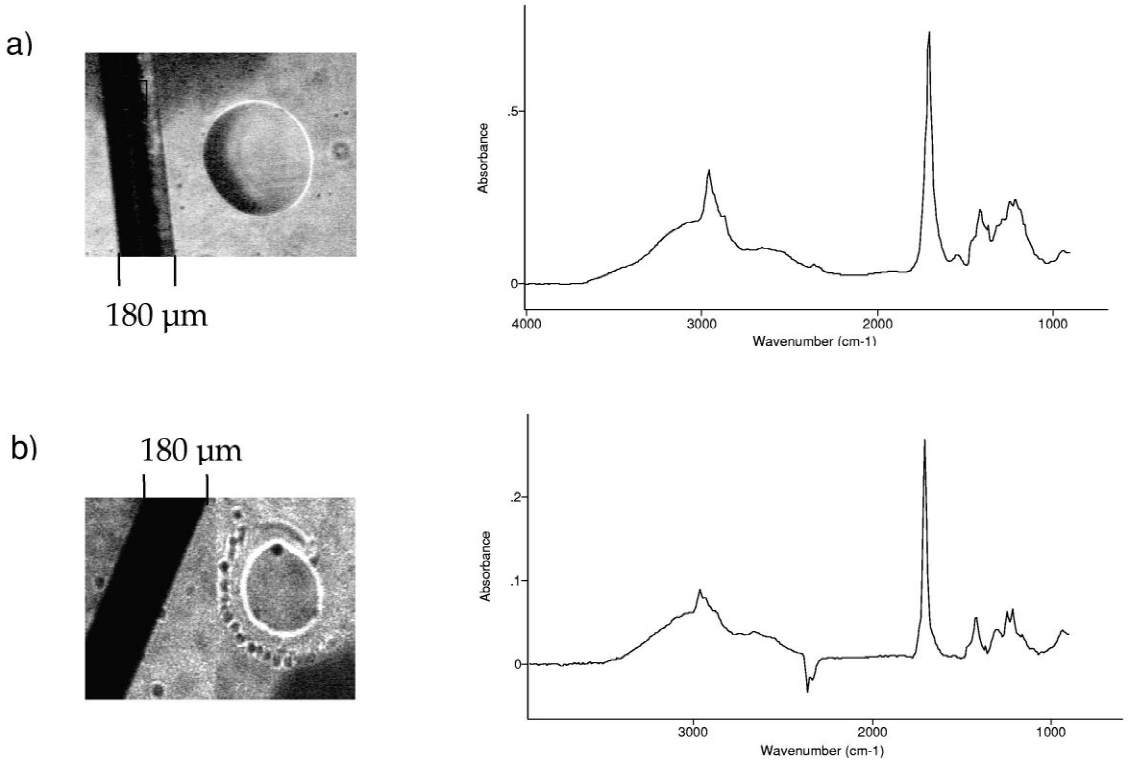

Fig. 3. Spot and spectra of pinic acid deposited on a CaF_2-slide corresponding to (a) 2.9 μg and (b) 290 ng of substance. For comparison of the spot size, a fused-silica capillary with an outer diameter of 180 μm was placed next to the spots.

conditions limit the use of the spray interface for LC–IR. Due to the strong gradient and the large amount of acetic acid, there was only a narrow range for all parameters that allowed adequate deposition conditions. As a result the deposition of the analytes was not successful in some experiments. One reason was that spray fluctuations caused by decarboxylation of acetic acid scattered the spots along the deposition line. Another problem was the hot spray jet itself, which caused evaporation of already deposited material. Because the main goal for the experiments was the identification of reaction products, the initial concentration was increased until enough material was deposited for detection. In Fig. 4 the UV-chromatogram of the gradient mixture is shown together with the picture of the spots.

Four major, well-separated spots were found on the slide. The corresponding spectra belong to the main compounds injected. The spectrum obtained from the small, hardly visible spot in front of *t*-NoPi might result from *cis*-norpinic acid. It does not differ significantly, though, from the spectrum obtained from the spot of *t*-NoPi. Pure standards for both isomers are not available. Thus, it is not possible to clearly assign the spectra to one isomer. In the case of pinic acid, only one spot was found, most likely consisting of both isomers. In the case of *c*-NoPi, the identification is ambiguous, since the corresponding spectrum does not differ significantly from the spectrum of *t*-NoPi.

4.4. Gas phase reaction of α-pinene with ozone

To demonstrate that it is possible to analyze terpenoids, which represent a class of compounds that is on the border between GC and LC volatility, from real samples with the LC–IR interface, we have obtained an extract from the gas phase reaction

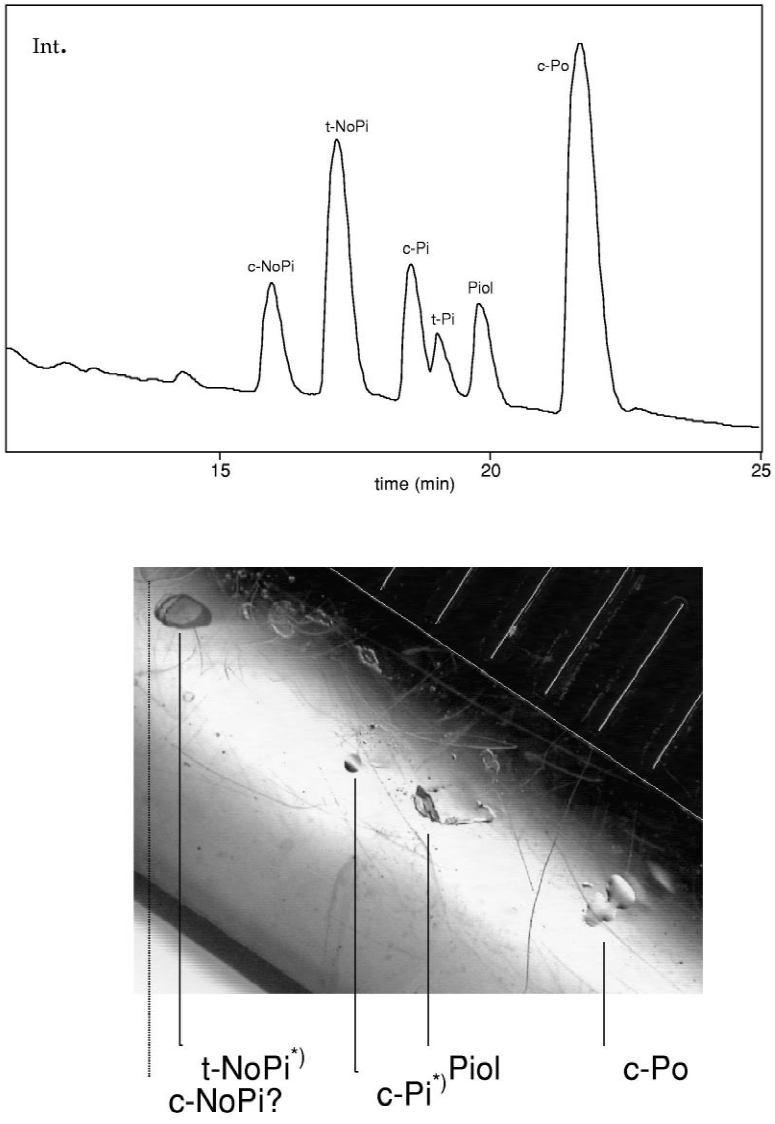

Fig. 4. Upper part: UV-chromatogram recorded at a wavelength of 210 nm. Lower part: Image of the spots found after solvent elimination on the ZnSe-slide. For comparison of the sizes, a ruler with a 1-mm scaling is placed next to the spots. The spots were marked according to the corresponding IR-spectra. *c*-NoPi: *cis*-norpinic acid; *t*-NoPi: *trans*-norpinic acid; *c*-Pi: *cis*-pinic acid; *t*-Pi: *trans*-pinic acid; Piol: pinolic acid; *c*-Po: *cis*-pinonic acid. In the case of *c*-NoPi, the identification is ambiguous, since the corresponding spectrum does not differ significantly from the spectrum of t-NoPi. *)Possibly both isomers.

between α-pinene and ozone. In Fig. 5 a microscopic image from a deposition slide is shown displaying the deposited compounds following chromatographic separation.

Due to the non-linear deposition that occurred because of the hot spray jet, the analytes were scattered along the deposition line. Therefore, two separate linescans have been used to detect the compounds of interest, indicated by the two lines in the picture. Fig. 6a and b shows an overview of the infrared spectra recorded along those two lines.

The infrared spectra allow us to assign the spots to

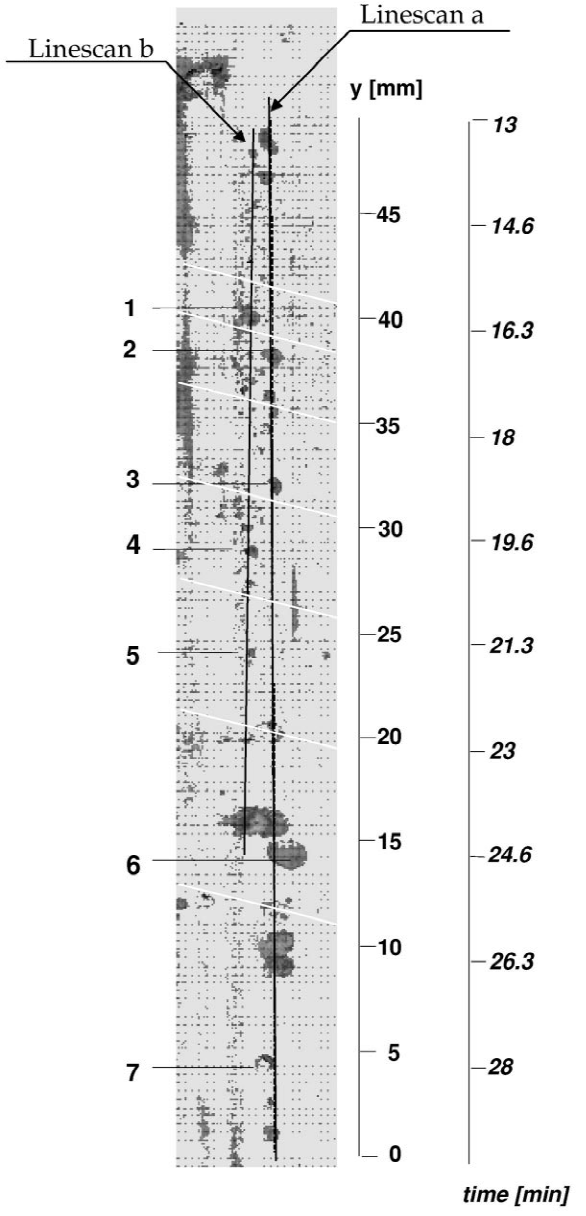

Fig. 5. Microscope picture of a deposition from an extract of the α-pinene–ozone reaction. The two lines in the picture indicate the lines along which the spectra were recorded.

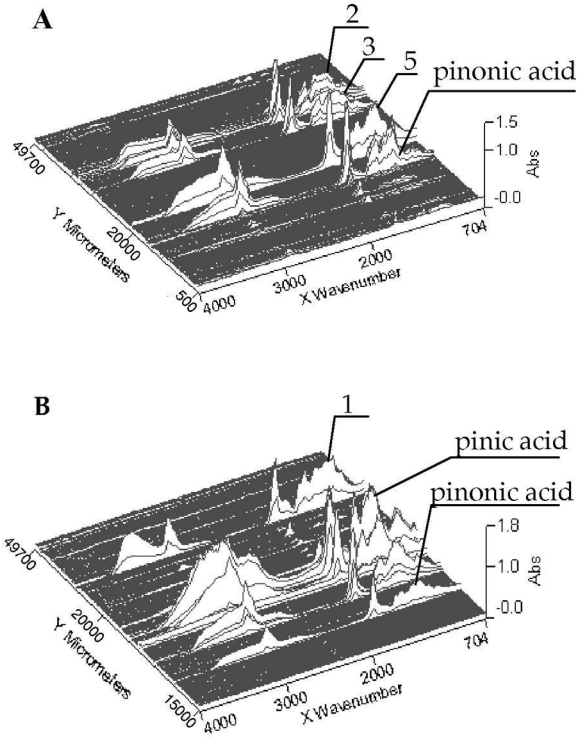

Fig. 6. (a) and (b) Three-dimensional views of the infrared spectra from the two linescans showing the signals detected along the lines indicated in Fig. 6. Numbers correspond to the deposition spot.

seven compounds that are products of the reaction. Spots 4 and 6 can be identified from available standard spectra as pinic acid and pinonic acid, respectively. Although the other spectra do not allow a conclusive identification of the compounds, some information about functional groups is possible. In Fig. 7a–e the spectra of the five remaining spots are shown.

Compound 1 (Fig. 7a) can be assigned with a primary alcohol, indicated by the bands at 3414 and 1055 cm^{-1}, respectively. Additionally, the characteristic bands of carboxylic acid dimers are present in the spectrum: a C=O-stretching vibration at 1712 cm^{-1} in combination with a broad dimer band around 2700 cm^{-1} and a dimer band at 1274 cm^{-1}. The frequencies at 2970, 2939, 2872 and 1374 cm^{-1} are characteristic for the C–H stretching and bending vibrations of CH_3- and CH_2-groups.

For compound 2 the spectrum (Fig. 7b) shows a very intense C=O-stretching vibration at a rather high frequency (1742 cm^{-1}). This indicates that apart from the carboxyl-group, which can be identified by a wide dimer band, an additional functional group containing a C=O double bond is present. The

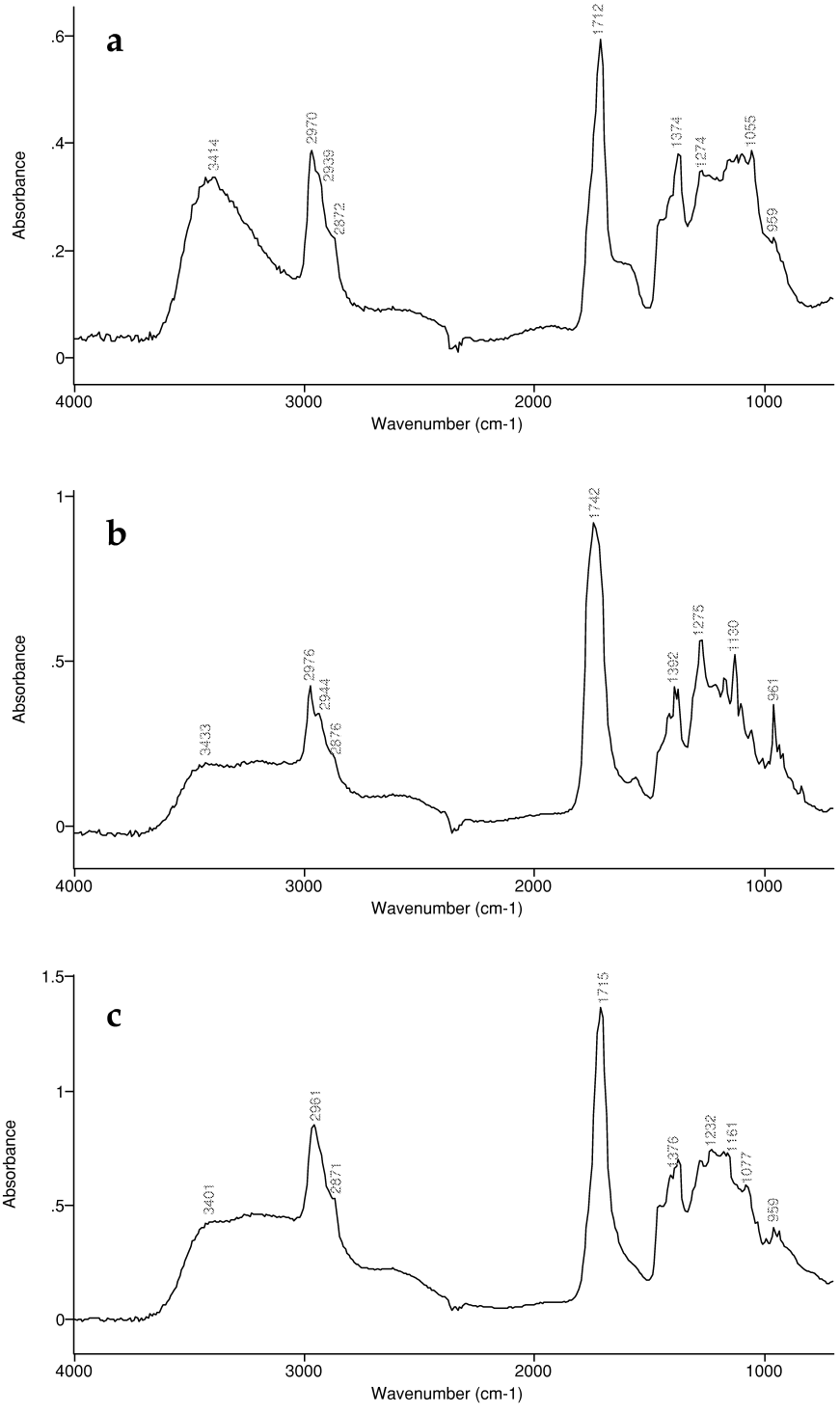

Fig. 7. (a)–(e) Infrared spectra obtained from spots 1, 2, 3, 5 and 7 respectively, of the deposited extract.

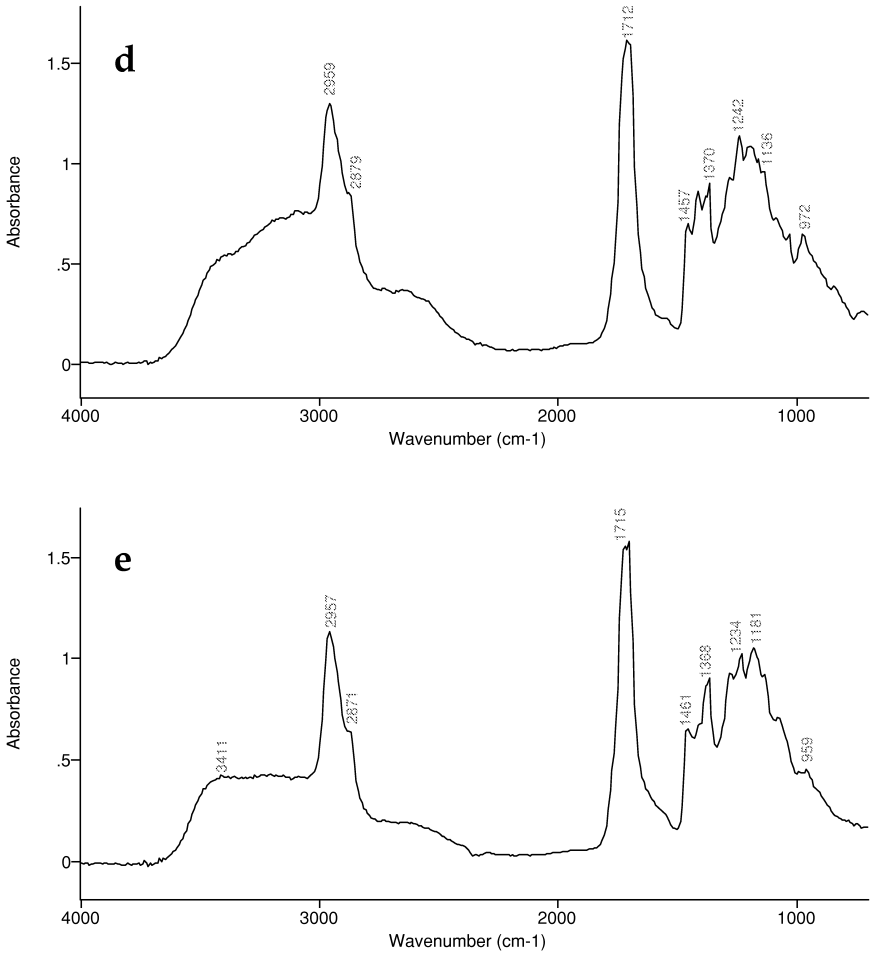

Fig. 7. (*continued*)

absorption band at 1275 cm^{-1}, which is too strong to result solely from the acidic dimer, in combination with the high frequency of the C=O-stretching vibration suggest the presence of an ester group. Since the bands of this group dominate the spectrum, it is difficult to clearly evaluate some of the other absorption bands.

Compound 3 (Fig. 7c) can also be identified as a carboxylic acid by the presence of the C=O band at 1715 cm^{-1} and the characteristic dimer bands in the IR-spectrum. Additionally, the bands at 2961, 2871 and 1376 cm^{-1} show the presence of CH_3- and CH_2-groups. The band at 3401 cm^{-1} is again rather weak, but could still indicate an OH-group corresponding to an alcohol. In the range of the C–O stretching vibrations, a couple of bands are present that could result from an alcohol.

The spectrum of compound 5 (Fig. 7d) only shows the characteristic frequencies of carboxylic acid dimers and of CH_3- and CH_2-groups. There is no indication for the presence of other functional groups. It cannot be excluded, though, that C=O-groups corresponding to ketones and aldehydes are present in the molecule, since they show absorption frequencies in the same range as carboxylic acids.

The spectrum of compound 7 (Fig. 7e) is very similar to compound 6 but clearly separated by chromatography and can also be identified by the IR-spectrum as a carboxylic acid containing CH_3- and CH_2-groups.

5. Conclusion

A sprayer interface for gradient LC–IR analyses was developed and is described. The experimental setup is simple and inexpensive, but allows us to analyze even compounds with boiling points just barely above the temperature of the heated sprayer. The experiments show that the critical parameter is not the sensitivity of infrared detection but the deposition process of the analyte, which is not easy and needs experience.

The first experiments concerning the observation and identification of reaction products formed during the α-pinene–ozone reaction are promising, where at least seven compounds are detected, though only two could be identified by spectra comparison as pinic acid and pinonic acid. Nonetheless, even though the structures of the compounds are related, resulting in similar spectra, LC–IR allows us to obtain important information about major oxygen containing functional groups for all detected compounds. Additional studies with other hyphenated techniques, e.g., LC–MS and even LC–NMR, may complement the results of the LC–IR analyses and help to identify the products formed during this reaction.

It has to be noted, that the determination of the low volatile reaction products is restricted, because the compounds have boiling points that are too close to the temperature necessary for the solvent elimination. The experiments show, that some compounds may even be partially evaporated after deposition on the slide. They can be removed by the hot spray and condensed outside of the spray line where it is cold enough, but where the spots can still be detected. To be able to obtain spectra of all compounds it was necessary to use higher initial concentrations of the analyte mixture.

Finally, it can be stated that LC–IR can supply valuable information and should be considered as an alternative and complementary method for the analysis of compounds that are not volatile enough for GC separation but almost too volatile for solvent elimination deposition.

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References

[1] P. Jackson, G. Dent, D. Carter, D.J. Schofield, J.M. Chalmers, T. Visser, M. Vredenbregt, J. High Res. Chromatogr. 16 (1993) 515.
[2] K.L. Norton, A.J. Lange, P.R. Griffiths, J. High Res. Chromatogr. 14 (1991) 225.
[3] W. Schrader, J. Geiger, T. Hoffmann, D. Klockow, E.H. Korte, J. Chromatogr. A 864 (1999) 299.
[4] T. Hoffmann, J.R. Odum, F. Bowman, D. Collins, D. Klockow, R.C. Flagan, J. Seinfeld, J. Atmos. Chem. 26 (1997) 189.
[5] R.M. Robertson, J.A. de Haseth, R.F. Browner, Appl. Spec. 44 (1) (1990) 8.
[6] C.C. Johnson, L.T. Taylor, Anal. Chem. 56 (1984) 2642.
[7] G.A. Luoma, R.D. Rowland, J. Chromatogr. Sci. 24 (1986) 210.
[8] S. Wachholz, H. Geissler, J. Bleck, J. Liq. Chromatogr. 11 (4) (1988) 779.
[9] D.E. Pivonka, K.M. Kirkland, Appl. Spec. 51 (6) (1997) 866.
[10] R. Vonach, B. Lendl, R. Kellner, Anal. Chem. 69 (20) (1997) 4286.
[11] J.J. Gagel, K. Biemann, Anal. Chem. 59 (1987) 1266.
[12] A.J. Lange, P.R. Griffiths, D.J.J. Fraser, Anal. Chem. 63 (1991) 782.
[13] A.J. Lange, P.R. Griffiths, Appl. Spec. 47 (4) (1993) 403.
[14] T.C. Schunk, S.T. Balke, P. Cheung, J. Chromatogr. A 661 (1994) 227.
[15] S. Bourne, Am. Lab. 30 (1998) 16.
[16] X. Liu, D.K. Ryan, Environ. Technol. 18 (1997) 417.
[17] A.M. Robertson, L. Wylie, D. Littlejohn, R.J. Watling, C.J. Dowle, Anal. Proc. 28 (1991) 8.
[18] A.M. Robertson, D. Littlejohn, M. Brown, C.J. Dowle, J. Chromatogr. 588 (1991) 15.
[19] G.W. Somsen, R.J. Vandenesse, C. Gooijer, U.A.Th. Brinkmann, N.H. Velthorst, T. Visser, P.R. Kootstra, A.P.J.M. Dejong, J. Chromatogr. 552 (1–2) (1991) 635.
[20] J.A. de Haseth, R.M. Robertson, Microchem. J. 40 (1) (1989) 77.
[21] R.M. Robertson, J.A. de Haseth, J.D. Kirk, R.F. Browner, Appl. Spectr. 42 (8) (1988) 1365.
[22] R.M. Robertson, J.A. de Haseth, R.F. Browner, Appl. Spectr. 44 (1) (1990) 8.

[23] R.C. Willoughby, R.F. Browner, Anal. Chem. 56 (14) (1984) 2626.
[24] V.E. Turula, J.A. de Haseth, Appl. Spectr. 48 (10) (1994) 1255.
[25] V.E. Turula, J.A. deHaseth, Anal. Chem. 68 (4) (1996) 629.
[26] J.A. deHaseth, V.E. Turula, Mikrochim. Acta [Suppl] 14 (1997) 109.
[27] G.W. Somsen, C. Gooijer, U.A.Th. Brinkman, N.H. Velthorst, T. Visser, Appl. Spectr. 46 (10) (1992) 1514.
[28] G.W. Somsen, E.J.E. Rozendom, C. Gooijer, N.H. Velthorst, U.A.Th. Brinkman, Analyst 121 (1996) 1069.
[29] S.L. Jordan, L.T. Taylor, J. Chromatogr. Sci. 35 (1) (1997) 7.
[30] G.W. Somsen, C. Gooijer, N.H. Velthorst, U.A.Th. Brinkmann, J. Chromatogr. A 811 (1998) 1.
[31] G.W. Somsen, C. Gooijer, U.A.Th. Brinkmann, J. Chromatogr. A 856 (1999) 213.
[32] T. Visser, M.J. Vredenbregt, G.J. ten Hove, A.P.J.M. de Jong, G.W. Somsen, Anal. Chim. Acta 342 (1997) 151.