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THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Section 2 **Test Methodology and Assessment**

Gas chromatographic determination of 1.8-Naphthalimide, N-Hydroxy-1.8-Naphthalimide (N,N-naphthaloylhydroxylamine) and the sodium salt of N-Hydroxy-1.8-Naphthalimide

by

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ABSTRACT

Gas chromatographic determination of 1.8-Naphthalimide, N-Hydroxy-1.8-Naphthalimide (N,N-naphthaloylhydroxylamine) and the sodium salt of N-Hydroxy-1.8-Naphthalimide

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A number of naphthalimide (NI) derivatives are used as efficient laser dyes, in medicine or in transmission electron microscopy. Only N,N-naphthaloylhydroxylamine (NHA) has been shown to be an effective wood preservative against wood decay fungi and termite damage.

However, limited information is available concerning the analytical detection of NI-derivatives in treated timber. There is a clear need for the analytical characterisation; e.g. with regard to the penetration depth or the assessment of retention after leaching.

This paper describes the development of a gas chromatographic method for determination of NI and their derivatives in timber. These investigations were carried out by means of direct thermal desorption-gas chromatography-mass spectrometry (TD-GS-MS) using the pure substances in solution, as well as direct analysis of treated southern yellow pine (SYP).

It was shown that the identification of NHA in treated SYP is possible using this analytical technique Furthermore first evidence is given for determining quantitative data.

Surprisingly, the chromatograms and especially mass spectra obtained for NHA and the sodium salt of NHA are identical to the mass spectra of NI.

The first results show that TD-GC-MS can be an option in determining the retention levels of NI and their derivatives in wood.

Key words (3-6): N-Hydroxy-1.8-Naphthalimide; N,N-Naphthaloylhydroxylamine; Gas chromatography; Wood preservative

1 INTRODUCTION

A number of 1.8-naphthalimide (NI) derivatives were synthesised and widely used as efficient laser dyes (PARDO et al. 1989), in biology and medicine (AVELINE et al. 1997, MIDDLETON and PARRICK 1985) or in calcium detection using transmission electron microscopy (ZECHMEISTER 1979).

However, only N,N-naphthaloylhydroxylamine (NHA) has been shown to be an effective wood preservative against wood decay fungi and termite damage (GREEN et al. 1997, CRAWFORD and GREEN 1999; GREEN et al 2002;).

To guarantee an efficient preservation of the treated timber a definite amount of the active ingredient(s) must be located in a certain penetration depth according the technical specification of the manufacturer. Therefore chemical analysis play an important role in internal and external quality control. Furthermore chemical analysis can aid by the interpretation of the mode of action.

In contrast to the analysis of inorganic wood preservative components the detection and quantification of organic actives in treated timber represents special problems due to small amount applied and their fixation. Nevertheless several analytical methods exist. A comprehensive compilation is given by SCHOKNECHT et al. 1998.

Thermal sample treatment methods such as pyrolysis or thermal desorption are used in combination with gas chromatography and mass spectrometry for the analysis of solids (HORN and MARUTZKY 1994, JÜNGEL et at. 2002).

Based on previous experiences (JÜNGEL et al. 2000) and similarities concerning the chemical composition of bis-(N-cyclohexyl-diazeniumdioxy)-copper (Cu-HDO) and Na-NHA analytical experiments were carried out with regard to the possibility of the detection of NI, NHA and Na-NHA in timber. The results obtained are presented in this paper.

2 EXPERIMENTAL

2.1 Material

The investigations were carried out using 1.8-Naphthalimide (NI, CAS 81-83-4), N,N-Naphthaloyl-hydroxylamine' (NHA or N-Hydroxy-1.8-naphthalimide, CAS 7797-81-1), the sodium salt of NHA (Na-NHA; CAS 6207-89-2) and southern yellow pine (SYP) blocks treated with Na-NHA (figure 1).

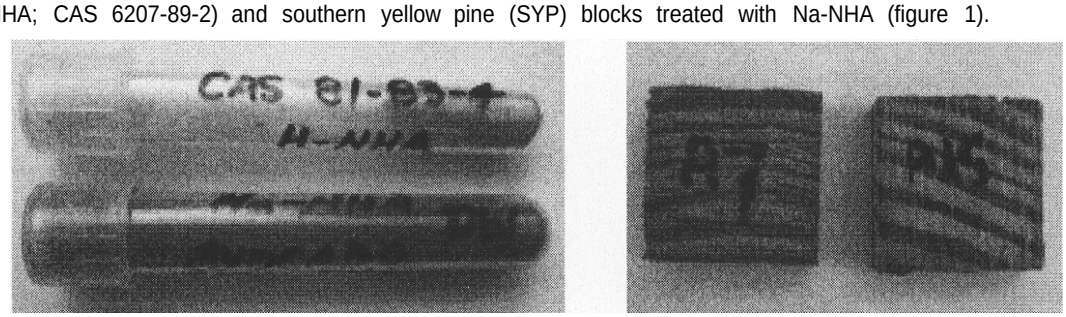

Figure 1: Illustration of the pure substances and the treated SYP

[1] This material was ordered from Sigma-Aldrich Co.

Solutions of the pure substances were made by means of “methanol for trace organic analysis” (Merck) containing definite different concentrations of NI, NHA or Na-NHA.

The impregnated SYP was stored in a standard climate for 4 weeks. At first the wood blocks were split mechanically. Afterwards the splits were milled using a type IKA A10 S laboratory mill to guarantee a nearly homogeneous distribution of the wood preservative. Finally the milled material was stored in a standard climate until the analysis was carried out.

2.2 Analytical parameter

The analysis itself was performed as described in JÜNGEL et al. (2000) by means of direct thermal desorption - gas chromatography - mass spectrometry (DTD-GC-MS). Due to the high melting points of the substances it was necessary to modify some of the settings of the thermal desorption (TD) unit as well as of the gas chromatographic and mass spectrometric method which are shown in table 1 and 2.

Table 1 : Settings of the TD unit

Temperature program:			
Initial temperature:		50°C	
Initial time:		30 s	
Ramp	Rate [°C/s]	ISO Temp. [°C]	ISO Time [s]
1	16	300	30

Carrier gas pressure program:				
Parameter	Step 1	Step 2		
Start pressure [psi]	8	8	18.5	8
Time [s]	30	30	15	240
End pressure [psi]	8	8	18.5	8

Table 2: Settings of the GC- and MS-method

Instrument Settings				
Ionisation Mode:			EI (+)	
Column type:			SGE BPX 35	
MS-method				
Mass spectroscopy:			Ion Trap	
Seconds per Scan:			0.50 s	
Source Temperature:			200°C	
Transfer Line:			275°C	
Start Time:			5.00 minutes	
Micro Scans:			4	
Scan Mode:			Full Scan: 50-250 SIM: 126,153,169,197	
GC Method –Oventemperature program:				
Initial value:			100°C	
Initial time:			30 s	
Ramp	Rate [°C/ min]	Final [degrees]	Hold [minutes]	Total [minutes]
1	20	300	2.50	13
2	0	300	0	13
3	0	300	0	13
4	0	300	0	13
GC Method-Injector pressure program:				
Initial value:			8 psi	
Initial time:			4 minutes	
Ramp	Rate [psi/ min]	Final [psi]	Hold [minutes]	Total [minutes]
1	2	16	0	8
2	6	24	1	10.33
3	4	30	2	13.83
4	0	30	0	13.83

3 RESULTS

3.1 Chromatographic investigation of solutions

In order to determine the retention time of the substances as well as their mass spectra in a first stage gas chromatographic measurements were performed by means of methanolic solutions containing 250ppm NI (figure 2), 250ppm NHA (figure 3) or 250ppm Na-NHA (figure 4).

Figure 2 illustrates that a sharp signal is recorded after a retention time of 9:06 minutes which should be NI. An evidence for this assumption is the corresponding mass spectra which contains four remarkable masses with 197, 169, 153 and 126. Whereas the first one fits with the molecular mass of NI, the other fragments can be derived according usual disintegration rules (McLAFFERTY and TURECEK 1995). For example the mass fragment 169 could be formed by a loss of carbon monoxide as a result of the bombardment. A further fragmentation or the total loss of the C_2HNO_2 group leads to m/z 126 corresponding to the naphthalene moiety.

It is well known that a direct database search is difficult due to the exact replication of spectra or that the database contain too few compounds (WARR 1993).

Nevertheless such a library search has been carried out. It is to be seen on the right side of figure that the mass spectra of 1H-Benz[de]isoquinoline-1,3(2H)-dione fits well with the MS of NI with regard to purity, fit as well as rfit. Furthermore it is obvious that the CAS number corresponds to that under test.

1H-Benz[de]isoquinoline-1,3(2H)-dione[2] is the name of NI according the Chemical Abstract nomenclature (MIDDLETON and PARRICK 1985) From these results it can be deduced that the identification of diluted NI is possible by means of DTD-GC-MS.

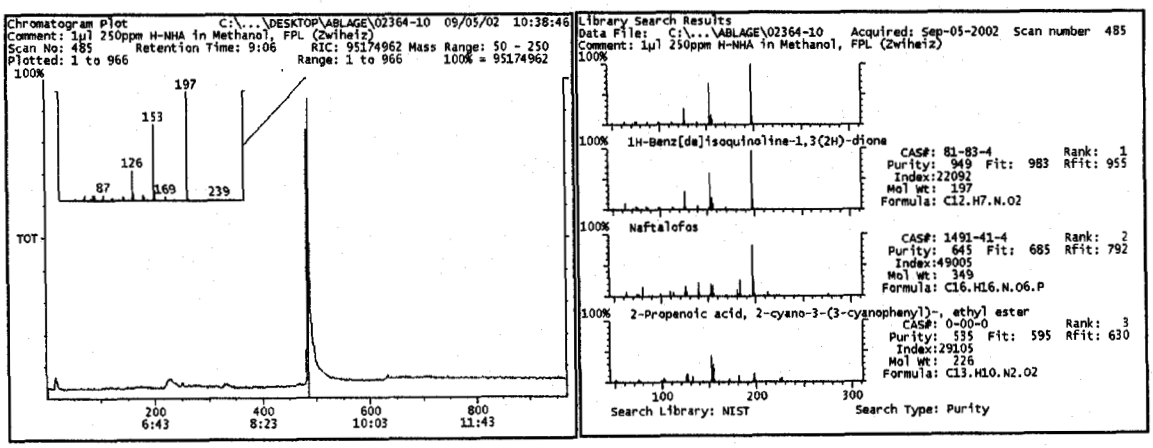

Figure 2: Methanolic solution containing 250ppm NI. Left: Full scan chromatogram and the mass spectra of NI. Right: Top 3 of the library search

[2] see also:http://webbook.nist.gov/cgi/cbook.cgi?ID=C81834&Units=SI&Mask=100#Mass-Spec

Surprisingly, the chromatograms and especially mass spectra obtained for NHA (figure 3) and the sodium salt of NHA (figure 4) are identical to the mass spectra of NI (figure 2).

Since the retention time of all these substances is identical this phenomenon can only be explained by a chemical reaction which took place within the thermal desorption unit during heating.

It is known that several hydroxylamines are characterised by a thermal instability (ROMPP 1995). On the other hand it is also described that NI shows an excellent performance to heating due to the so-called self-association (SZADOWSKI et al. 1978).

Considering these aspects it is to assume that during the thermal desorption process an elimination of oxygen took place followed by a rearrangement of the hydrogen atom.

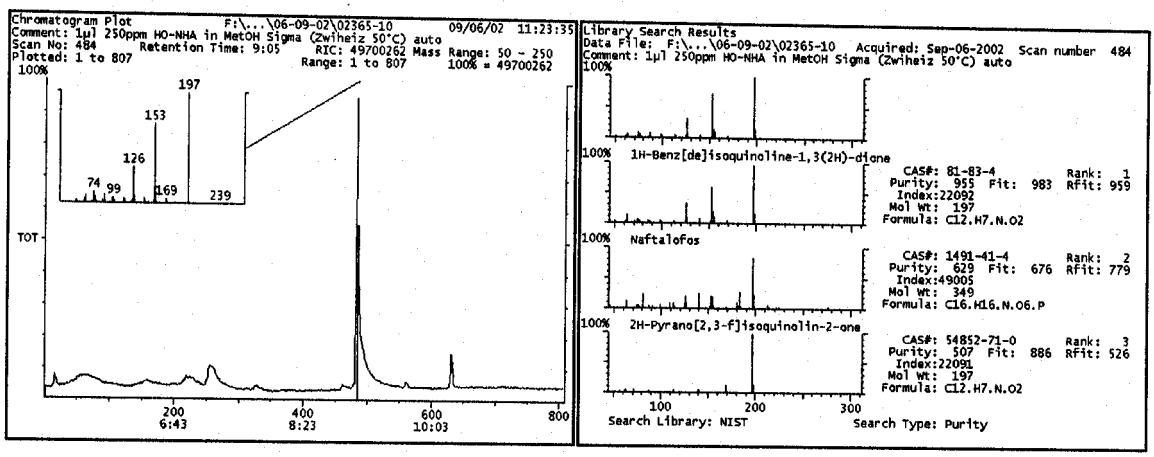

Figure 3: Methanolic solution containing 250ppm NHA. Left: Full scan chromatogram and the mass spectra obtained for NHA. Right: Top 3 of the library search

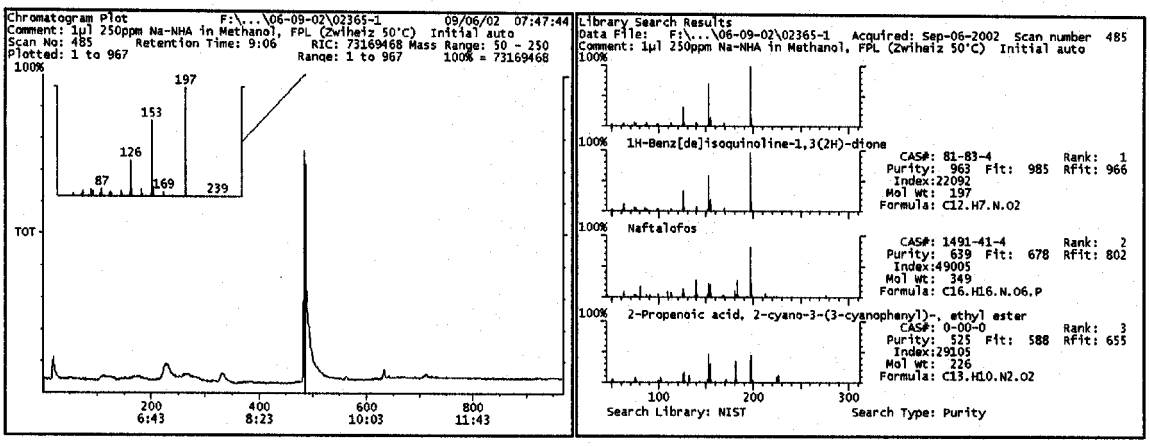

Figure 4: Methanolic solution containing 250ppm Na-NHA. Left: Full scan chromatogram and the mass spectra obtained for Na-NHA. Right: Top 3 of the library search

From the obtained results it can be derived that a distinction is not possible between NI and NHA using this technique at the moment.

3.2 Determination of the reproducibility

The reproducibility of the gas chromatographic method was evaluated by means of methanolic solutions containing 100ppm and 250ppm NI, NHA or Na-NHA. Each solution was measured at least 4 times. The evaluation itself based on a quantification using the height and area of the molecular peak. As an example some of the obtained results for N,N-naphthaloylhydroxylamine are shown in figure 5 and 6.

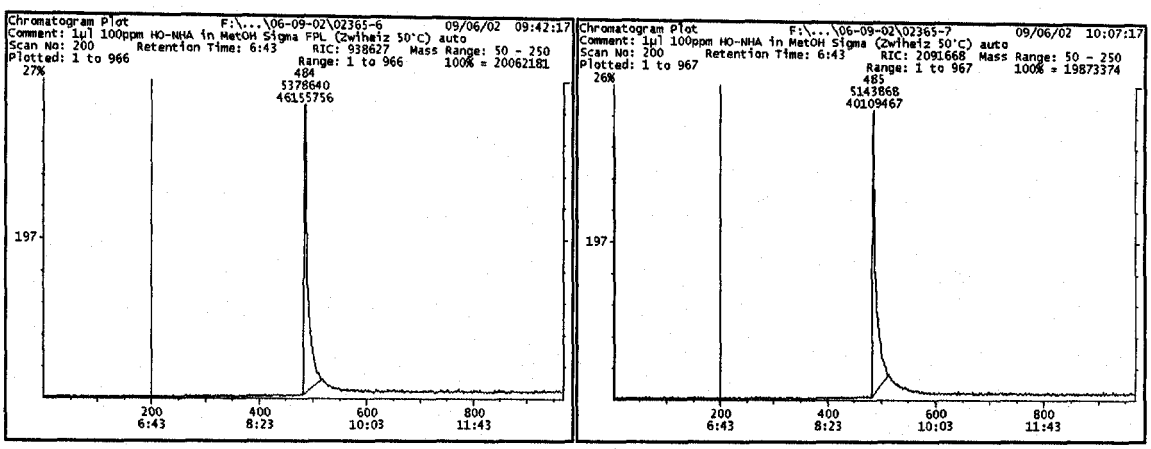

Figure 5: Quantification on the basis of the molecular mass using solutions containing 100ppm N,N-naphthaloythydroxylamine

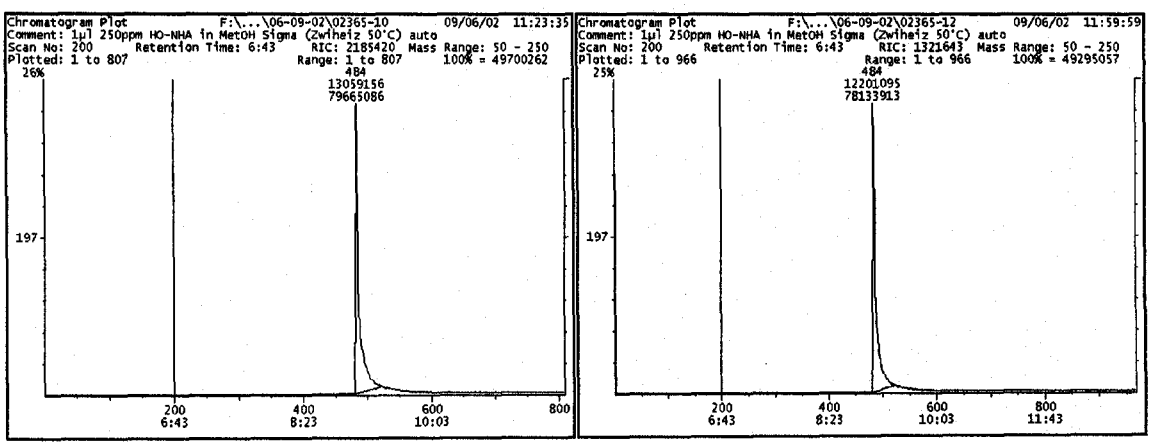

Figure 6: Quantification on the basis of the molecular mass using solutions containing 250ppm N,N-naphthaloyl hydroxylamine

It is clearly to be seen that an increase of the NHA concentration from 100ppm to 250ppm leads to an approximate doubling of the signal intensity. The concentration of 250ppm seems to be near at most measurable content since a further increase of the concentration (500ppm) results in saturation effects. Similar data were obtained for solutions containing NI or Na-NHA.

3.3 Gas chromatographic investigation of treated SYP

For qualitative (figure 7) and semi-quantitative (figure 8 and 9) investigations of treated SYP a definite amount of Na-NHA containing milled SYP was used.

As expected a number of signals were obtained in the case of investigations of treated SYP whereby the majority result from the timber itself. The active could be clearly identified by means of the corresponding mass spectra although the separation of desorbed substances is wrong (figure 7; left). Furthermore the left figure shows an extreme noise indicating that already a partial pyrolysis of the timber took place. Since the FSC is expressed as a SIC not only the number of signals is reduced but also the base line is minimised. Such a chromatogram would be suitable for a quantification.

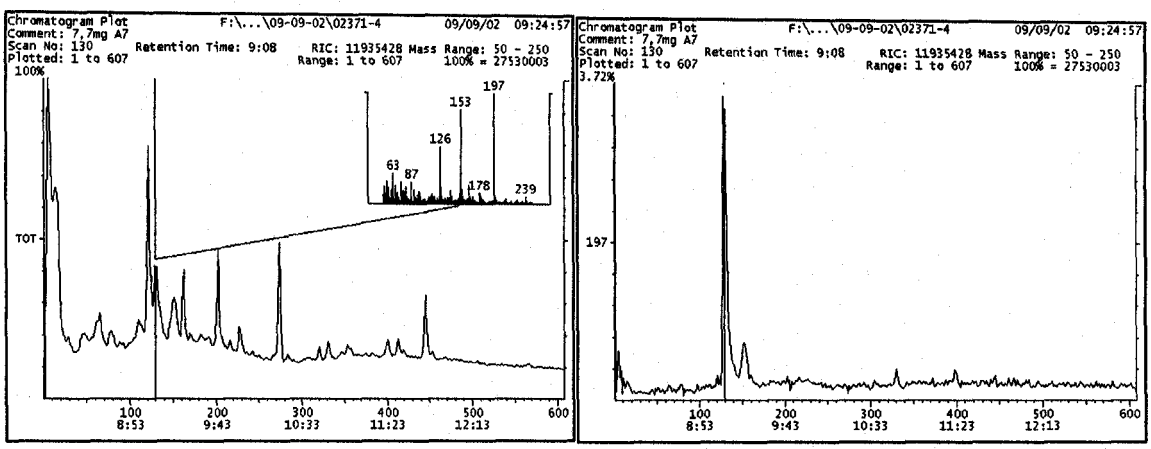

Figure 7: Southern yellow pine treated with Na-NHA. Left: Full scan chromatogram (FSC) and the mass spectra of NI. Right: Selected ion chromatogram (SIC) for the molecular mass.

In contrast to qualitative investigations of treated timber the quantification of an active requires a higher expenditure. In case of chromatographic measurements it is common practice that a quantification is carried out using the height and/or the area of a certain peak.

Since the original retention level of the samples was unknown the “quantification” was performed indirect via reproducibility. Figures 8 and 9 illustrate that the differences of the signal intensities are acceptable between identical samples considering the inhomogeneous distribution of the active.

The major problem at this stadium is that no continuous direct GC-MS investigations of treated SYP can be carried out due to contamination of the pre-column and within the thermal desorption unit as a result of the adsorption of carbon-like pyrolysis products (figure 10).

The investigation of extracts obtained from treated SYP might be an interim solution because during the measurements of methanolic solutions containing NI derivatives such an effect could not be observed.

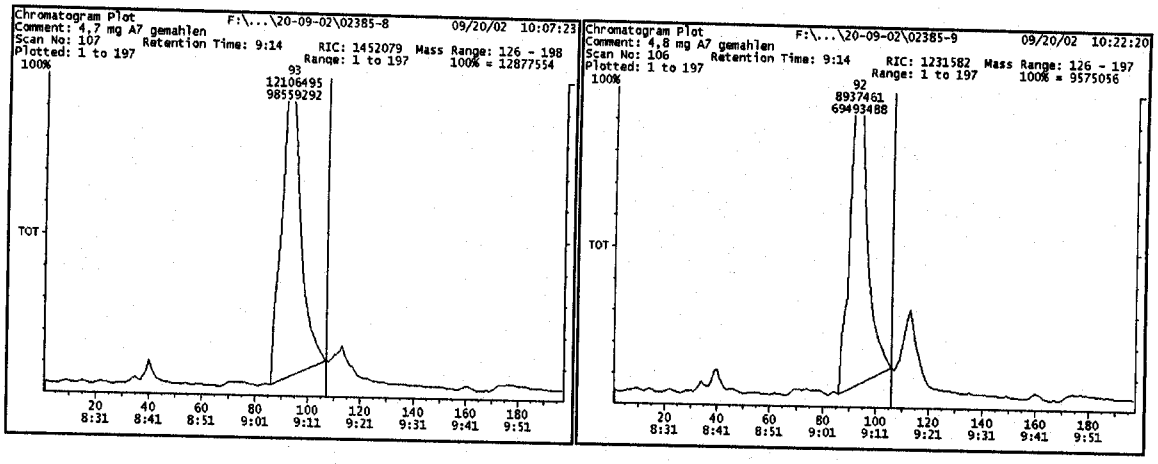

Figure 8: Southern yellow pine treated with Na-NHA. Left: Quantification on the basis of the molecular mass using 4,7 mg treated SYP (A7). Right: Quantification on the basis of the molecular mass using 4,8 mg treated SYP (A7).

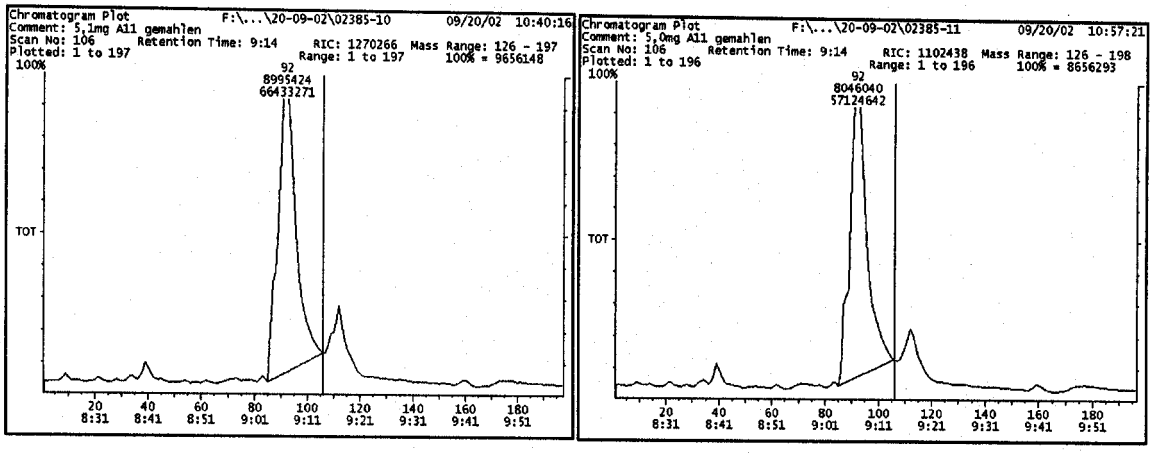

Figure 9: Southern yellow pine treated with Na-NHA. Left: Quantification on the basis of the molecular mass using 5,1 mg treated SYP (All). Right: Quantification on the basis of the molecular mass using 5,0 mg treated SYP (AI 1).

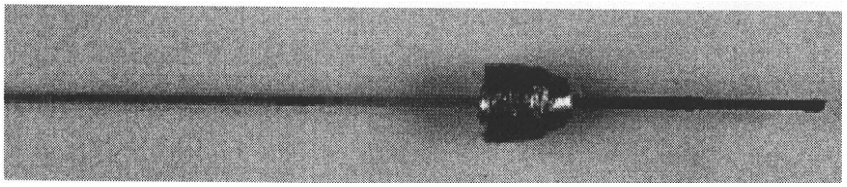

Figure 10: Contamination of the pre-column after approx. 15 measurements of treated SYP

4 CONCLUSIONS

The results concerning the analytical determination of NI, NHA and Na-NHA in different phases are based on DTD-GC-MS. It could be shown that the investigation of these substances result in identical chromatograms as well as mass spectra.

The molecular peak of NI with 197 is a recommendable mass for the analysis of such substances as it allows the quantification up to concentrations of 250ppm NHA in solution.

Direct investigations of treated SYP should be performed in a selected ion mode using the masses 197, 169, 153 and 126. However, a limiting factor is at the moment the contamination due to the adsorption of pyrolysis products within capillary pre-column as well as in the thermal desorption unit. Therefore it is recommended to perform an extraction at first and then to investigate the extract by means of DTD-GC-MS. Such a strategy would allow to carry out continuous measurements.

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