Development of Naphthenic Acid Fractionation with Supercritical Fluid Extraction for use in Wood Decay Testing

Brett Niemi[1], Wayne St. John[1], Bessie Woodward[2], Rodney DeGroot[2], and Gary McGinnis[1]

[1]Institute of Wood Research, Michigan Technological University, Houghton, MI
[2]Forest Products Laboratory, USDA Forest Service, Madison, WI

Abstract: In recent years, the performance of copper naphthenate as a wood preservative has been in question. To understand the varying results of copper naphthenate in preventing wood decay, a closer look at eight naphthenic acid (NA) supplies was undertaken. Initial studies of NA samples from individual suppliers revealed large differences in chemical composition and wood preservation ability. Samples were rated from best to worst determined by percent weight loss due to fungal activity. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that one of the intermediate quality wood preservatives from our wood decay studies contains a large portion of phenolic and aliphatic impurities in addition to the naphthenic acid components. To determine the effect of the impurities on the efficacy studies, we developed a method using supercritical fluid extraction (SFE) to remove them from the sample without changing the characteristics of the naphthenic acid composition. Comparison of NA SFE extracts continues.

Background

Copper naphthenate has been used as a wood preservative since the 1940's, but has not been used as extensively as other preservatives such as pentachlorophenol, creosote, and chromated copper arsenate (CCA). Growing concerns about the health and environmental effects of these widely used preservatives have fostered a search for alternatives. This research has renewed interest in copper naphthenate. To fully develop the use of copper naphthenate as a wood preservative, it is necessary to have a full understanding of naphthenic acid stock materials.

NA, as commercially derived from crude oil, is a complex mixture of monocarboxylic acids containing one or more alkyl-substituted alicyclic rings (naphthenes), with lesser amounts of aliphatic carboxylic acids [1]. Few, or no, aromatic compounds are present in pure supplies. NA's have long been important as biomarkers and geochemical indicators for petroleum operations [2]. The ability of NA to complex copper, forming copper naphthenate, increased interest in this product to be an effective and environmentally safe wood preservative as opposed to traditional treatments [3]. Recently, however, the efficacy of copper naphthenate as a wood decay fungicide has been suspect due to inconsistencies among commercial supplies of NA [4]. Therefore it is important to develop methods to evaluate and control batches of NA from different sources.

The compositional heterogeneity of NA makes it difficult to characterize by common chromatographic and/or spectroscopic techniques. There are thousands of alicyclic acids present in most naphthenic acid supplies. Even synthetic supplies may contain substantial variations; some may contain substantial quantities of mineral and crude oils and fatty acids. Even supplies from a single manufacturer or feed stock are often significantly different in composition [4]. NA consumers traditionally evaluate their naphthenic acid supplies by acid number (via titratable acids), but this value can be erroneous due to impurities with acid functionalities.

Attempts to characterize NA by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) have been reported [4-8]. Although some procedures are more useful for identification of impurities in NA, two GC-MS methods were developed for identification of naphthenic acid components [7,8]. Nuclear magnetic resonance data has been used to determine methylene/methyl ratios and aromatic content of NA samples, but the technique

does not provide structural information on complex mixtures without extensive sample fractionation [9].

Introduction

To understand the varying results of copper naphthenate in preventing wood decay, a close look at eight NA supplies was undertaken [7]. Initial studies of naphthenic acid samples from individual suppliers revealed large differences in chemical composition and wood preservation ability. Wood decay studies indicate #4 as an intermediate quality wood preservative (in the group studied) and GC-MS analysis reveals that NA #4 contains a large portion of phenolic compounds in addition to the naphthenic acid components. To determine the effect of the impurities on the efficacy studies, a method needed to be developed to isolate the impurities without changing the characteristics of the naphthenic acid composition.

Supercritical fluid extraction (SFE) techniques were used to fractionate naphthenic acid #4 for use in fungal decay studies by means of laboratory soil-block method. Supercritical carbon dioxide was used to remove the phenolic fraction of NA #4 to produce two isolated components; (1) NA (with some residual impurities) and (2) the impurities removed from NA #4. Isolation of the two components was necessary to produce three samples for efficacy testing: (1) NA with decreased phenolic levels, (2) NA #4 with increased phenolic levels, (3) and the original NA #4. By testing these three samples, the wood preservation effects of the phenolic components in NA #4 will be determined. In order to prepare samples with large enough volume (approximately 20 mL) for fungal decay studies, preparative scale extractions were necessary.

Materials and Methods

Reagents

Eight individual stocks of NA were acquired for our study (supplier's identity not revealed due to confidentiality). Toluene was used as the solvent for applying NA onto the wood blocks. Liquid carbon dioxide (bone dry, Interstate-Valweld Inc., Marquette, MI) was used as the extraction solvent in the SFE. Dichloromethane (GC resolved grade, Fisher Scientific) was used to prepare naphthenic acid standards and for extraction from SFE media. Methyl alcohol (HPLC grade, Fisher, Pittsburgh, PA) was used as the trapping media for the SFE procedure. NA #4 was derivitized in order to analyze the NA content in the sample [7]. Tertiary-butyldimethylsilyl derivatizations for NA analysis were prepared with N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide which contained 1% t- butyldimethylsilyl-chloride (MTBSTFA, Regis Technologies, Inc., Morton Grove, IL).

Decay Tests

Soil-block decay tests were conducted following the guidelines of AWPA E10-91 [10]. All blocks were 19 by 19 by 19 mm (3/4 in3) southern pine sapwood. Each of the eight NA stocks was diluted with toluene to yield a series of six treating solutions of 100, 50, 25, 12, and 6 percent NA stock (w/w - NA stock/toluene). Two controls were used: untreated blocks and blocks treated with toluene. Three replicate blocks were used with each control and with each treating solution of each stock. Blocks weighed an average of 3.5 grams prior to treatment. The average gain in weight due to treatment ranged from 3.5 grams with toluene to 3.6 grams with the stock solutions. The treated blocks were allowed to air dry in a fume hood for three weeks after treatment. They were then equilibrated at 26°C and 30% relative humidity. Blocks were steam sterilized for 20 minutes and cooled prior to inserting them into decay jars.

Treated blocks were challenged with the brown rot fungus, *Postia placenta* (Madison 698). The loaded soil blocks were incubated at 26°C and 70% relative humidity for 12 weeks. Visual observations of fungal growth over the treated blocks were made at 4, 8, and 12 weeks after insertion into the soil jars. At the conclusion of the incubation period, blocks were removed from the decay jars, re-equilibrated and reweighed. The amount of decay that occurred was expressed as percent weight loss.

Chemical Analysis

All samples were analyzed by a Hewlett Packard 5890 Series II GC equipped with a Hewlett Packard 5971 mass selective detector (MSD) and a 30 M x 0.25 mm I.D., 0.5 um film DB-5MS capillary column (J&W Scientific, Inc.; Folsom, CA). Characterization of NA samples was performed by injecting 1 μL of 2000 ppm sample via splitless injection. Characterization of NA components in samples was done by the method developed by St. John et al. [7]. The MSD was operated in the electron impact ionization mode (70 eV). Since the underivitized and derivitized samples contained different components, two oven programs were used to optimize separation. Analysis of underivitized samples had a column temperature program of 3 minutes at 40°C followed by a 5°C/-minute ramp to 100°C and subsequently by a 10°C/-minute ramp to 300°C. Derivitized samples were then heated to 60°C for 20 minutes to generate the t-BDMS derivative [7]. Analysis of derivitized samples had a column temperature program of 3 minutes at 120°C and an 8°C/minute ramp to 310°C (held for 10 minutes). The GC-MS transfer line was operated at 310°C. The instrument was auto-tuned using perfluoro-tributylamine and operated at 1.2 scans/sec from m/z of 70-550. The MSD remained off for the initial 3 minutes of the run to allow solvent to pass through the system.

Fractionation

A supercritical fluid extractor consisting of an Isco 260D syringe pump and Varian GC oven was used for all SFE extractions. The SFE oven temperature was 40°C and carbon dioxide pressure was 1200 psi. Average flow rate of liquid carbon dioxide was 1.0-1.5 mL/minute during each 480 minute extraction. The matrix was washed with distilled/deionized water, oven dried at 120°C, and sieved to remove dust particles before being introduced to sample. Approximately 7 mL of NA #4 was spiked onto 10 g diatomaceous earth matrix and placed into a stainless steel cell for each extraction. The extraction was preformed 4 times on separate NA #4 samples to produce enough sample needed for fungal decay studies. Effluent carbon dioxide gas was bubbled through 50 mL of collection solvent (methanol) which was replaced every 60 to 100 minutes while the syringe pump was being refilled with carbon dioxide. After the supercritical fluid extraction, the extraction cell contents were desorbed with 200 mL of dichloromethane and the solvent was evaporated under a gentle stream of nitrogen. All collection solvent portions were pooled together, concentrated to 5 mL, and added to 30 mL of NA #4.

Results/Discussion

Fungal Decay Results

After four weeks of soil-block culture testing, very little difference between all eight NA samples was observed (table #1). In the 3 w/w% blocks, fungal colonization was observed in NA samples #4, #5, #6, and #7. Treatments of 6, 12, 25, 50, and 100 w/w% for all NA samples were effective against visual fungal growth at this time.

As seen in Table 1, some differences between NA samples were visually observed after eight weeks. The lowest treatment of 3 w/w% showed evidence of fungal colonization for all eight NA samples. However, at this concentration samples #1, #2, #3, and #8 seem to provide better protection against colonization than #4, #5, #6, and #7. At treatments of 6 w/w%, only NA samples #2 and #4 prevented fungal growth. At treatments of 12-50 w/w% NA samples #5 and #7(except at 25 w/w%) failed to protect the wood blocks from fungal colonization.

The blocks were removed from the soil bottles and brushed free of mycelium after twelve weeks. Each block was allowed to air dry for two days before being placed in a 26°C and 30% relative humidity chamber for equilibration. After four weeks, percent weight loss was determined for each block (Table 2). The eight NA samples treated at 3 w/w% exhibited significant weight loss. In Table 3, the eight samples are ranked according to efficacy of fungal decay protection.

A closer look at the impurities found in NA #4 shows a large content of substituted phenols and saturated straight-chain hydrocarbons. Several substituted phenols and C10-C20 were identified in the sample via match with National Institute of Standards and Technology (NIST) mass spectral library. Due to the complexity of the NA #4 TIC, it

is nearly impossible to separate and identify each component in the sample. In order to make simple generalizations of structural identification in the complex TIC, a time profile from 18 to 33 minutes (spectra A-E, each averaged over 3 minutes) of averaged ion peaks was generated (Figure 2). As time increases in the TIC, the average mass spectrum changes from predominantly phenolic to straight chain hydrocarbon fragmentation ion patterns. Spectrum A contains large ion peaks at m/z 122, 121 and 107, common fragment ions of phenols substituted with aliphatic groups. Common phenolic ion peaks m/z 135 and 121 are joined with other predominant ions m/z 95, 81, 69, 57 and 41 in spectrum B to create a confusing averaged spectra. Spectra C, D, and E show signs of typical long saturated straight-chain fragmentation with clustered peaks 14 mass units apart at m/z 43, 57, 71 and 85 along with a smooth sloping decrease in mass unit intensity at each ion cluster.

NA #4 was chosen to be fractionated by SFE because it contains a relatively high impurity content and it performed moderately in preventing fungal decay. Comparisons of the mass spectra of three samples generated by the SFE fractionation are seen in Figure 3. The region from 14 to 24 minutes in extracted sample TIC has been greatly reduced compared to the original NA #4 TIC. The three fractions generated by SFE have the same NA content (by ion mass) as seen in Figure 4.

Summary and Future Direction

The eight NA samples were evaluated via soil-block laboratory test. With respect to the weight loss data, NA #1 was found to be the best in preventing fungal decay by Postia placenta, whereas #7 was the least effective. Each of the eight NA samples was analyzed by GC-MS for impurity content; most were significantly different in composition. One sample (NA #4) was chosen for fractionation by SFE. The fractionation produced three samples for future fungal decay testing: (1) NA #4, (2) NA #4 with decreased impurities, and (3) NA #4 with increased impurities. Each of these three samples was analyzed to ensure that the NA composition was not changed by the fractionation procedure. Once these three samples (and three others based on another NA sample currently being fractionated) are evaluated by the soil-block laboratory test, we will have a better understanding of what determines the efficacy of NA supplies in preventing fungal decay of wood.

Acknowledgements

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DISCUSSION

GARY MCGINNIS: Thank you for having me here. I really appreciate the opportunity to attend the AWPA meeting this year. Thank you.

SESSION CHAIRMAN PRESTON: Thanks, Gary. Are there any questions from the audience?

JEFFREY J. MORRELL, OREGON STATE UNIVERSITY: Gary, I enjoyed your paper. I am curious as to why you chose to take commercial formulations and break them down instead of, perhaps, taking pure naphthenic acid and materials to it to see how they affected performance?

DR. MCGINNIS: That's a good question. I think a lot of the concern and a lot of the work has been why there were huge differences between the different naphthenic acids. Originally what we wanted to do was to look at the formulations and to develop the GCMS method. As we got more and more involved in it, we it's much easier to identify the impurities in here and remove them this way. Because of the various differences in efficacy we wanted to find out why, for example, in some instances we find large numbers of phenols and why that made such a huge difference,. It seemed to be easier to go this way than add materials to it. Secondly, it is very difficult to say what is 100% naphthenic acid. We're not sure at any of these, they are such complex mixtures. I think the only one that was close was probably about 70, or 75%, naphthenic acid. We don't know what is a pure naphthenic acid right now.

SESSION CHAIRMAN PRESTON: Are there any more questions?

JAMES BRIENT; MERICHEM COMPANY: After you extracted out the phenolic compounds, using your supercritical fluid extraction, did you get any data on the soil block efficacy test. Did I miss that, or is that still under way?

DR. MCGINNIS: That's still on the way. We fractionated two different commercial types of naphthenic acid and the soil blocks on the fractionated materials and on the combined materials are being done right now. That should be finished in about six months.

MR. BRIENT: Second one is a comment on your gas chromatograms, a lot of the spikes on top of the naphthenic acid hump, are actually the fatty acids. They're not impurities. They're just naturally occurring fatty acids. Just thought I'd bring that to your attention.

DR. MCGINNIS: That's a possibility. We can tell what they are, but exactly where they come from, we can't. Although sometimes you can look at the natural abundance and see if they're typical of what you would find, or not.

SESSION CHAIRMAN PRESTON: Are there any more questions? If not, I'd like to thank Gary, one more time.

This concludes the portion of the program in honor of Bob Arsenault. I'd like to thank Bob Inwards for his introduction and the speakers for all of their presentations. I particularly want to thank Barbara Arsenault for her attendance here this morning.

Our next speaker is Jeff Slahor. He's a graduate of the University of Maine. He's currently a Research Instructor at the Appalachian Hardwood Center at West Virginia University and his research is primarily on the preservative treatment of hardwoods and related areas. His paper is entitled "Treatability of Five Appalachian Wood Species with Creosote and Timbor." I welcome Jeff to the podium.

Table 1. Visual obervations of fungal growth on NA treated blocks at 4 and 8 weeks in soild-block test with *Postia placenta,* (n=3)

NA	100 w/w%		50 w/w%		25 w/w%		12 w/w%		6 w/w%		3 w/w%	
sample	4 wks	8 wks	4 wks	8 wks	4 wks	8 wks	4 wks	8 wks	4 wks	8 wks	4 wks	8 wks
1												
2												
3												
4												
5												
6												
7												
8												

	Blocks sparsely covered with fungal growth
	Blocks mostly covered with fungal growth

Table 2. Percent weight loss after twelve week soil-block test with *Postia placenta,* (n=3)

sample	100 w/w%	50 w/w%	25 w/w%	12 w/w%	6 w/w%	3 w/w%	0 w/w%
1	1.61	2.86	1.89	1.43	1.26	3.96	
2	3.85	2.83	2.07	1.51	1.14	4.74	
3	3.34	2.00	2.46	1.31	1.15	5.18	
4	2.89	2.11	1.58	0.99	0.64	6.11	
5	0.68	2.77	1.19	1.02	3.25	17.61	
6	1.05	2.88	2.57	0.61	1.35	6.76	
7	6.58	2.18	2.05	0.43	3.92	45.98	
8	2.63	2.33	2.12	0.19	0.39	13.22	
Toluene							53.23
Control							59.74

Table 3. Rank of fungal decay suppresion on wood samples based on percent weight loss

Rank	naphthenic acid sample
1 (best)	1
2	2
3	3
4	4
5	6
6	8
7	5
8 (worst)	7

GC-MS Identification and Fractionation of Impurities

Each NA sample was analyzed by GC-MS to generate a "fingerprint" of the various chemical components present in each sample. All eight total ion chromatograms (TIC) of the naphthenic acid samples in Figure 1, reveal significant differences in actual composition.

Figure 1. TIC's of the eight NA's used for soil-block testing.

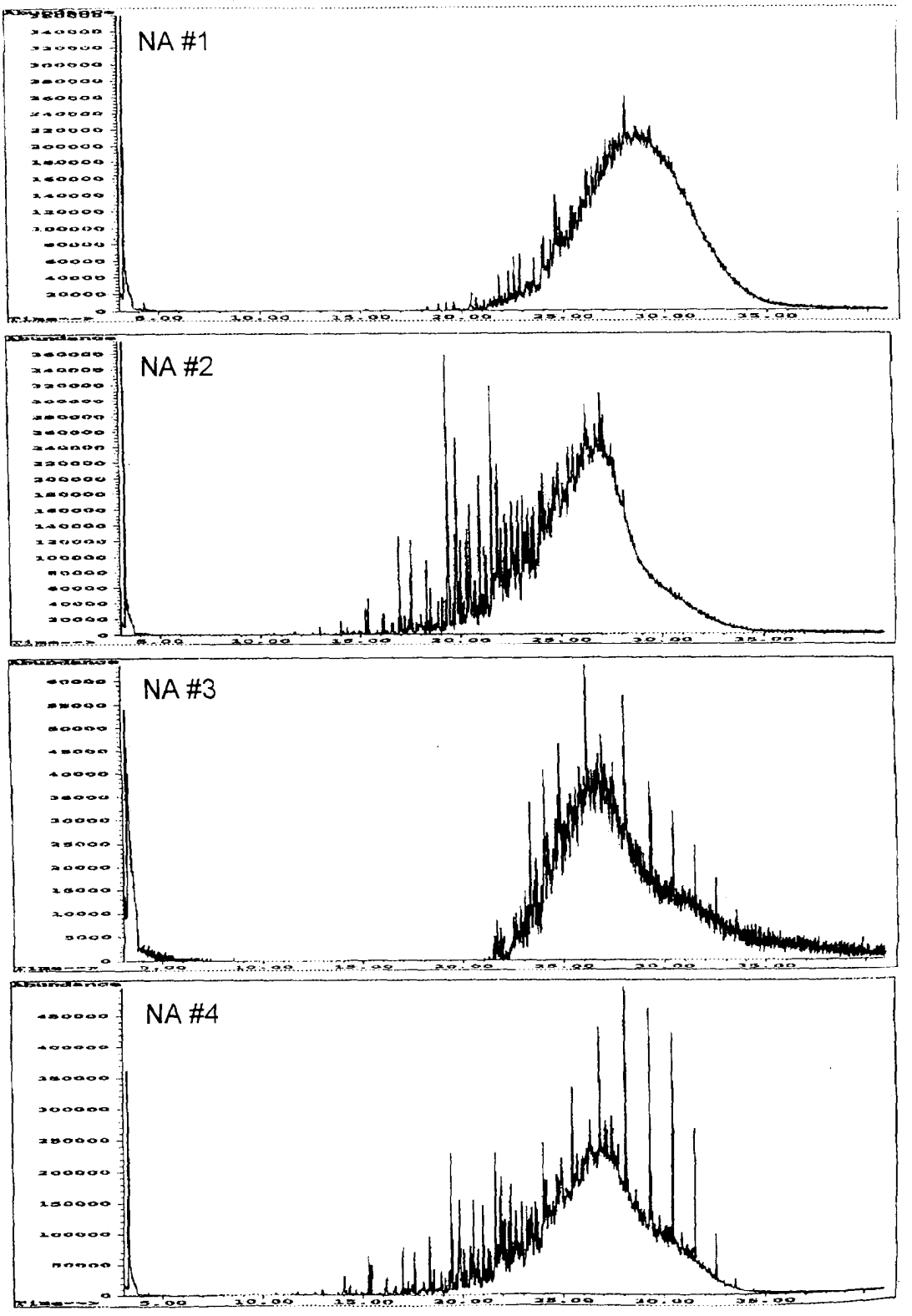


Figure 2, continued.

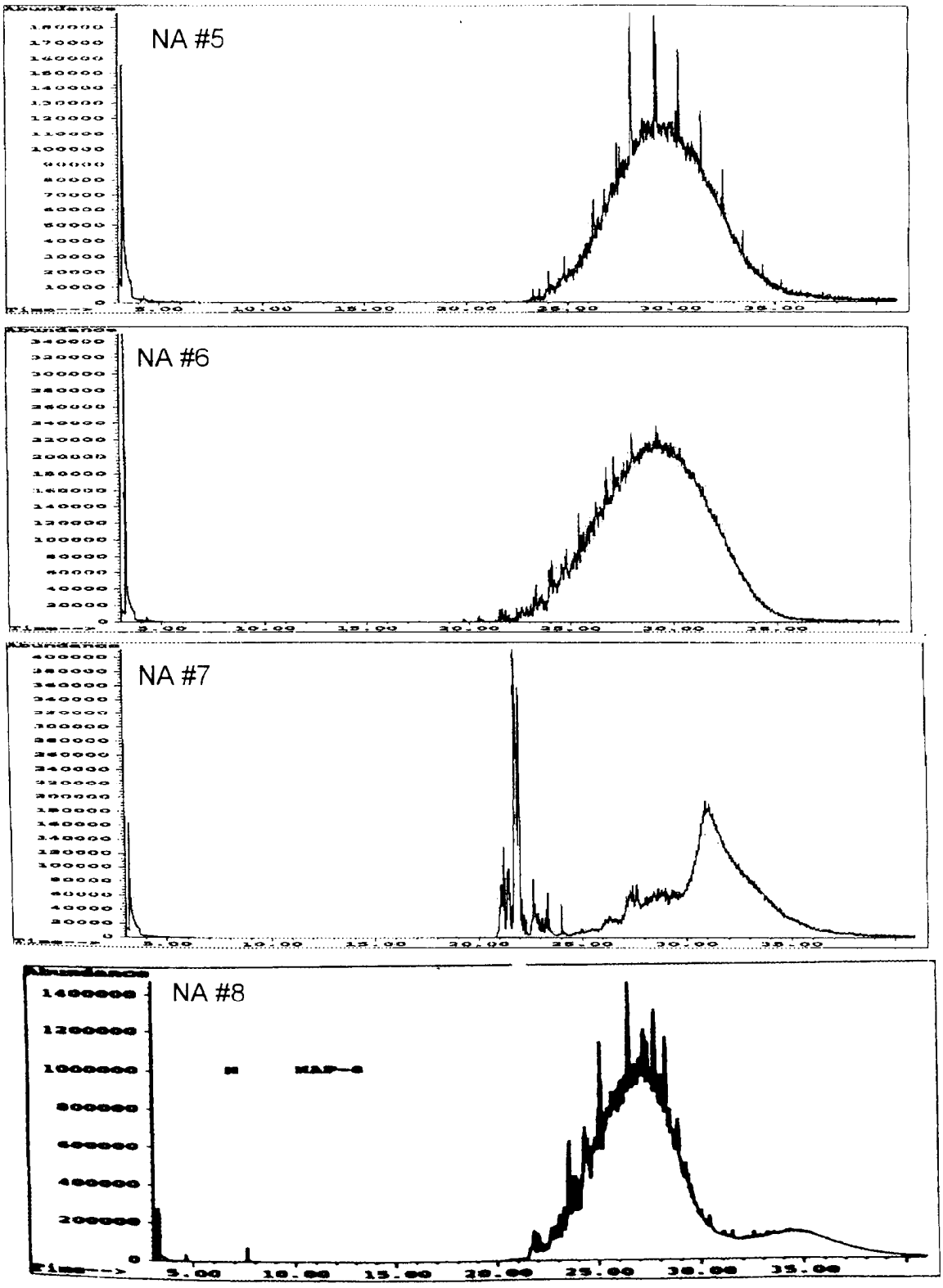
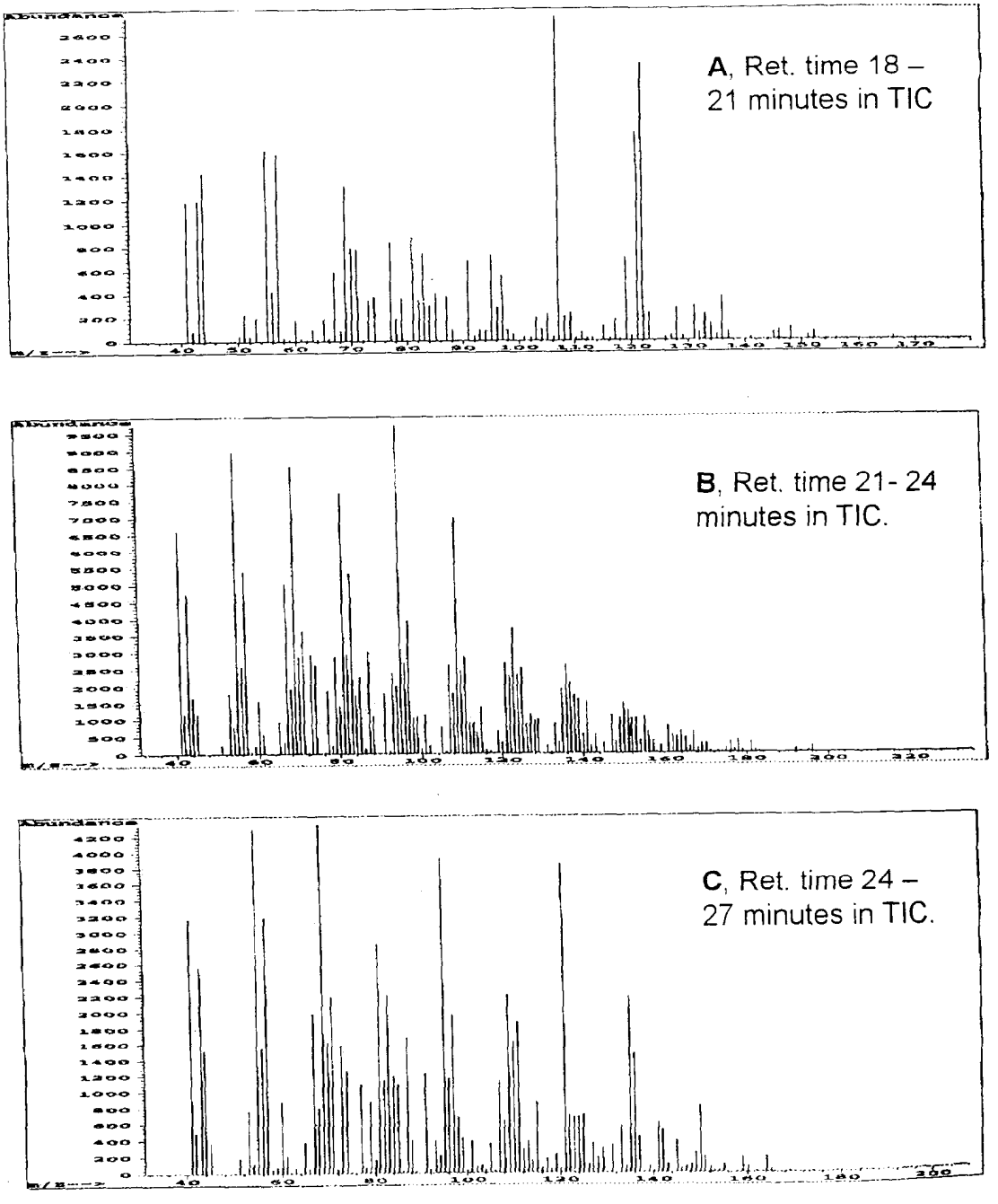


Figure 2. Time profile of average ion mass in NA #4 (note difference in m/z scales for each spectrum.



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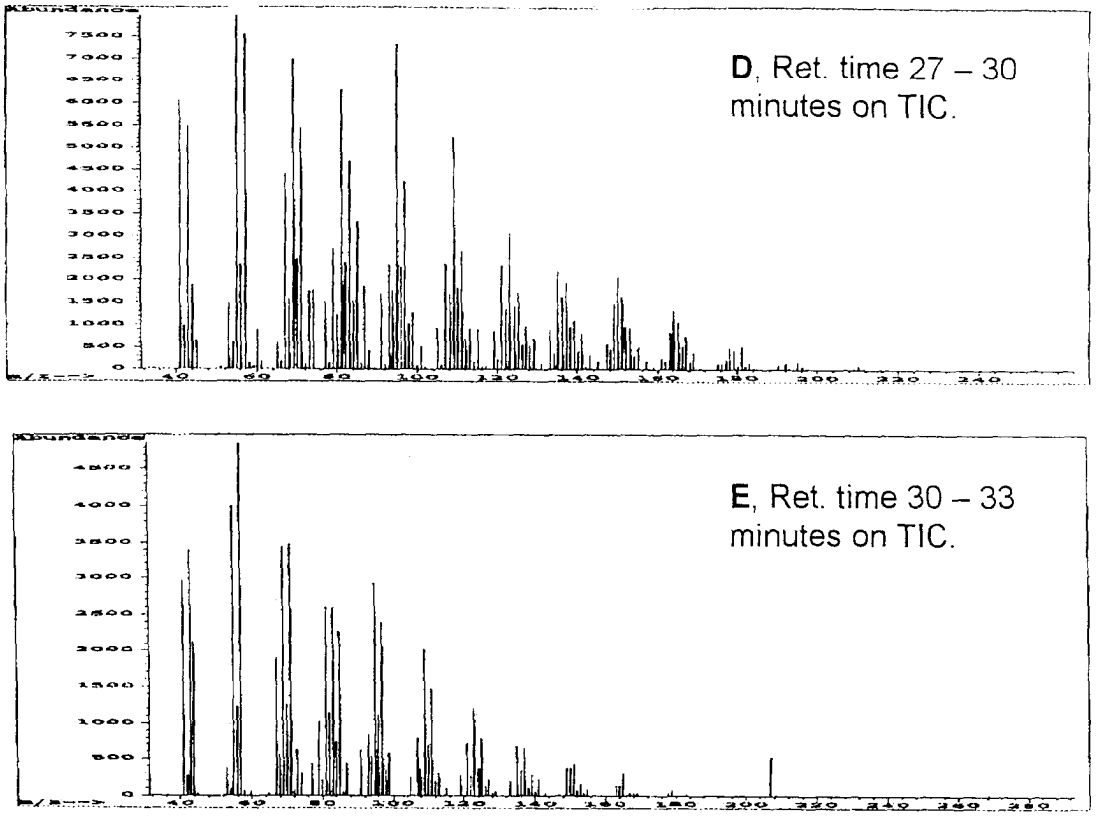
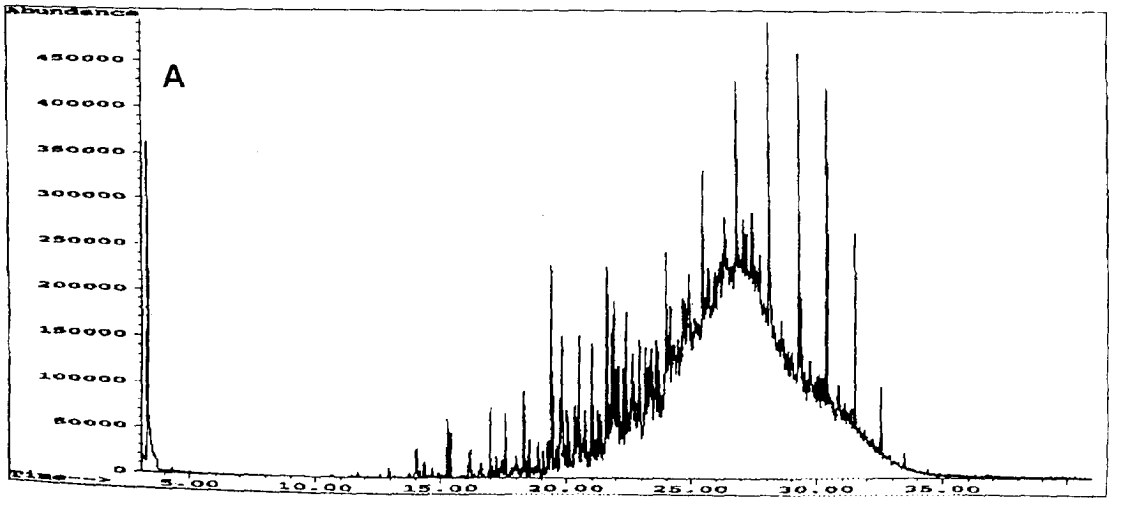


Figure 3. NA #4 samples before fractionation (A), minus extracted impurities (B), and with added impurities (C).



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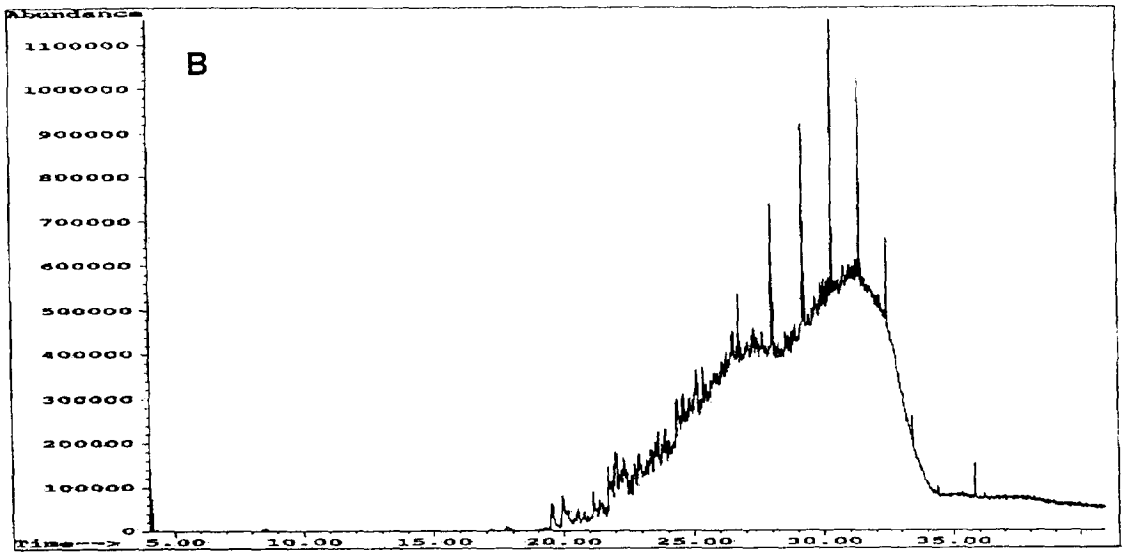


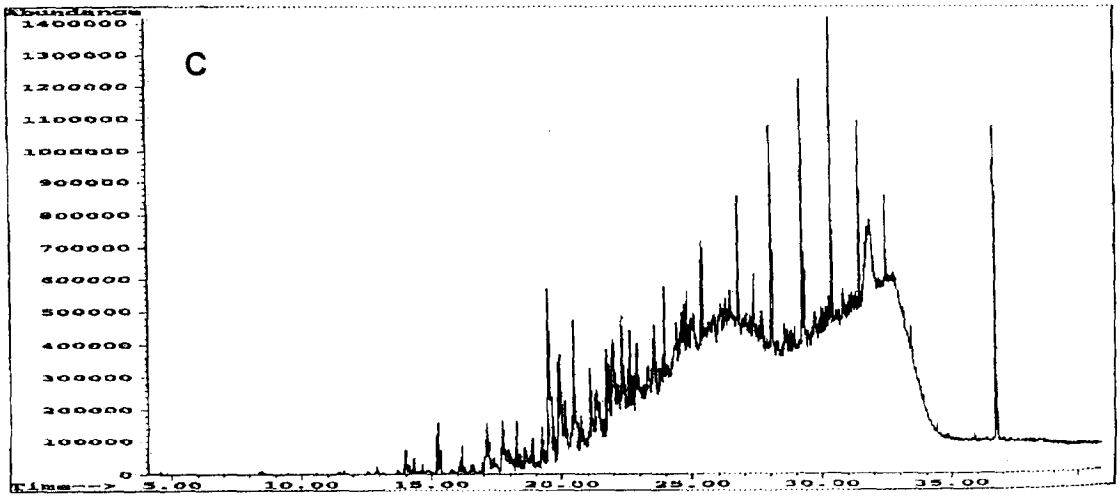
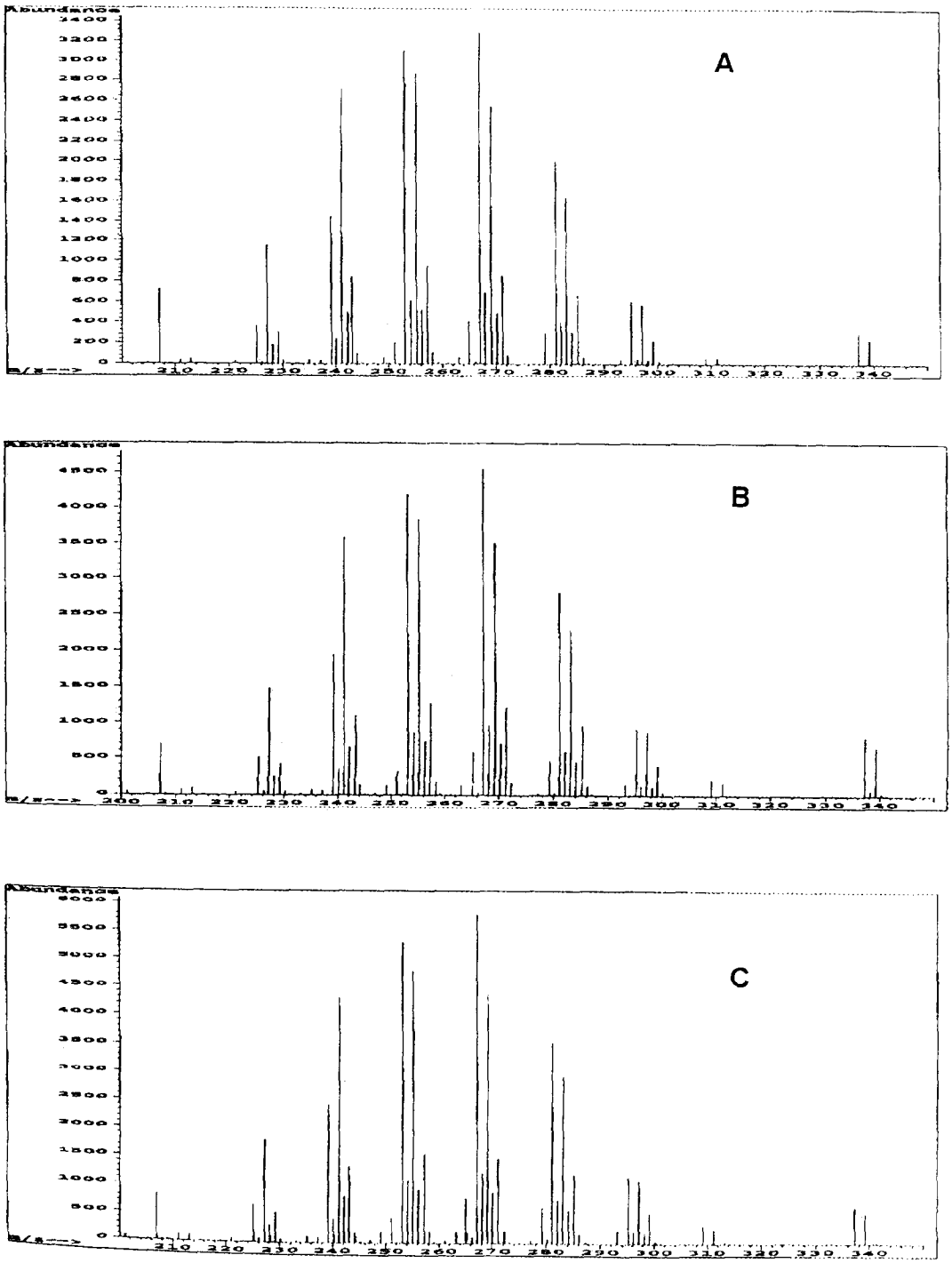


Figure 4. NA characterization in NA #4 (A), NA #4 after SFE (B), and NA #4 with added impurities (C).



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