



# Identification of Nicotine by Gas Chromatography/Mass Spectroscopy Analysis of Smoking Pipe Residue

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Ethnographic sources show the spiritual importance of tobacco in Native American Societies. Archaeological evidence, such as Early Woodland Period smoking pipes, indicate that this spiritual function has been maintained for thousands of years. However, ethnobotanical research on the prehistory of tobacco smoking in Eastern North America has been hampered by a lack of direct evidence prior to the Middle Woodland Period. Research involving a gas chromatographic/mass spectrographic technique (GC/MS) addresses the problem of identifying tobacco through the analysis of pipe residue. Results point to a possible Early Woodland Period use of tobacco in the Eastern Woodlands.

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## Smoking Pipes and the History of Tobacco

**E**thnographic and ethnohistoric documentation indicates that smoking pipes and tobacco have long been important in the ritual lives of Native Americans (Brown, 1989; Springer, 1981). Pipe ceremonies today serve to validate and sanctify social organization and group activities (Furst & Furst, 1983; Kaiser, 1984; Paper, 1987, 1988, 1992; Pego *et al.*, 1995; Steinmetz, 1984). Archaeological recovery of smoking pipes in ancient burials is evidence that the “Smoking Complex” has considerable temporal depth (Springer, 1981; von Gernet, 1992, 1995; von Gernet & Timmons, 1985). From simple tubular pipes in the Late Archaic of the second millennium BC, finely crafted tubular pipes were developed and became widely associated with Early Woodland cultures in the Eastern Woodlands of North America. The best known are “blocked-end” tubes found in Adena burial mounds. This article presents chemical evidence that these early smoking pipes were used to ingest nicotine.

There has to date been comparatively little insight into the early history of smoking. The earliest evidence for the ingestion of nicotine in the form of *Nicotiana* sp. tobacco comes from Middle Woodland period sites near the confluence of the Illinois and Mississippi Rivers, dating between *c.* 1850–1750 BP, or AD 100–200 (Asch, 1991, 1994: 45; Haberman, 1984: 271; Stafford & Sant, 1985: 356; Wagner, 2001; Winter, 2001). Dates for the Great Plains, Southwest, Southeast and lower Great Lakes, fall between the 5th and 8th centuries AD (Asch & Asch, 1985: 196; Haberman, 1984: 272–273;

Wagner, 1998: 840). The oldest known dates for tobacco are from the North Coast of Peru, with dates ranging between 2500 and 1800 BC (Pearsall, 1992: 178). Tobacco was probably first domesticated in South and Central America. After its initial domestication, the practice of cultivating tobacco spread throughout North America, though the specific geographic route by which this occurred is at present unknown (Asch, 1994; Asch & Asch, 1985: 195; Ford, 1981; Haberman, 1984: 274–277; Pearsall, 1992; Wagner, 1998, 2001). By the time of Native American Contact with Europeans, tobacco cultivation was commonplace (Asch, 1994: 46).

While smoking pipes recovered from Early Woodland contexts are evidence for smoking, they are only indirect evidence for the use of tobacco (Haberman, 1984). Ethnographic and ethnohistoric research has shown that Native Americans in the Northeast smoked numerous plant species besides tobacco, including dogwood bark (*Cornus* sp.), juniper bark (*Juniperus* sp.), sumac leaves (*Rhus glabra*), or bearberry leaves (*Arctostaphylos uva-ursa*) (Brown, 1989: 313; Springer, 1981: 220; Yarnell, 1964). The fact of what specifically was smoked in Early Woodland pipes has remained elusive.

What has been needed is a means of testing for the presence of psychoactive chemical compounds using the pipes themselves. A promising form of such evidence, the residue left behind when the actual plant material is burned, has seen little systematic research. This article presents a technique for the chemical identification of nicotine alkaloid from pipe residue, and summarizes current results.

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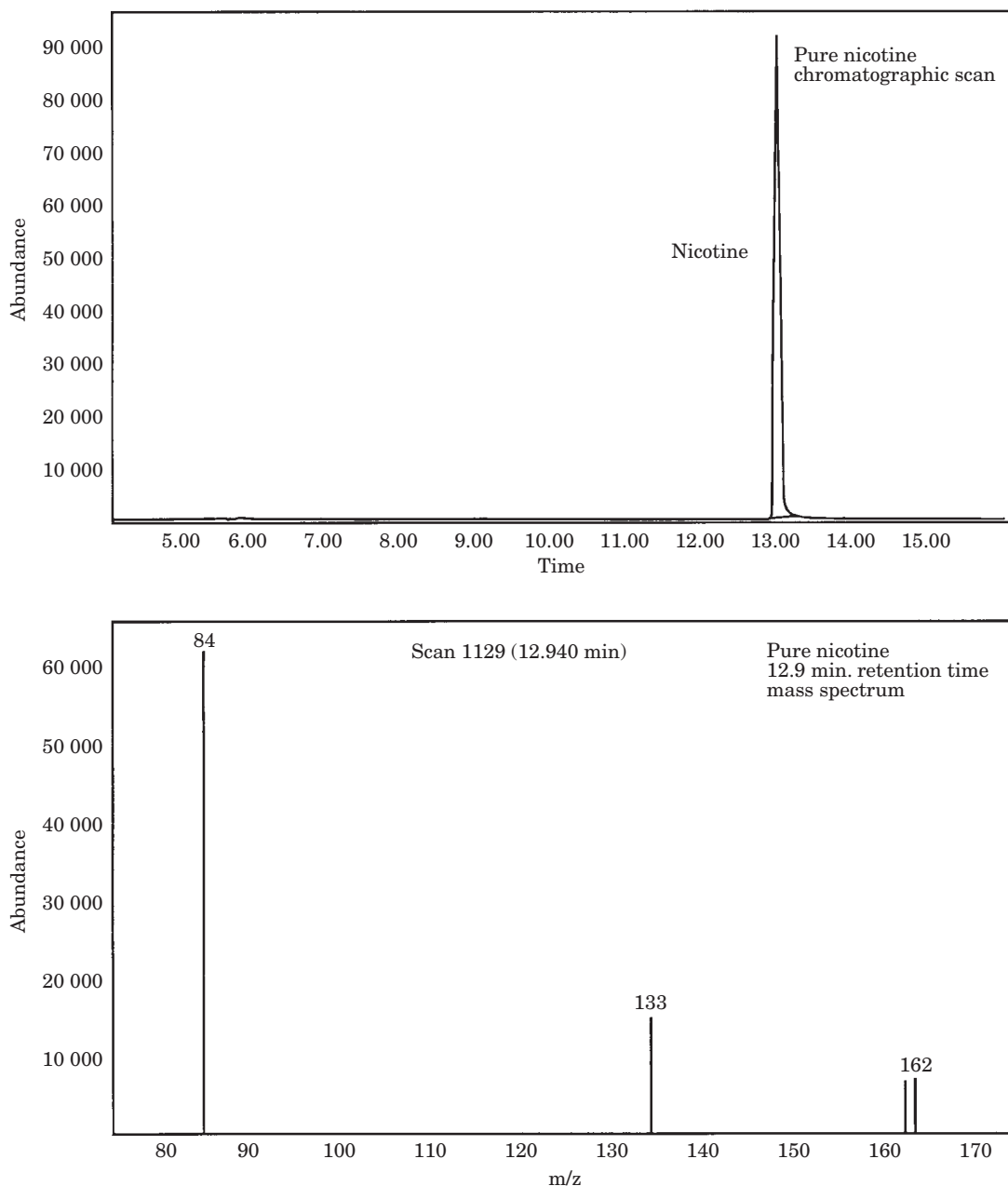


Figure 1. Gas chromatogram/mass spectrum for pure nicotine in methanol solution.

### Residue Analysis and Smoking Pipes

While systematic research on identifying tobacco through residue analysis is a recent undertaking, the idea of using chemical means is not new. Chemical analysis was used on residue from Basket-maker pipes in 1922 (Dixon & Stetson, 1922) and focused on the identification of the principal active alkaloid of *Nicotiana* tobacco, nicotine. Nicotine was never identified at that time. Later researchers assumed that

nicotine was unlikely to survive in archaeological contexts long enough to be of use as a chemical marker (Fletcher, 1979; Haberman, 1984). One reason cited was that nicotine was not likely to survive the process of combustion in the pipe (Fletcher, 1979; Knight, 1975). Were this the case, the nicotine contained in tobacco would not be transmitted to the smoker, and there would therefore be no physiological effect from smoking. Other hypotheses that leaching by groundwater would draw nicotine away in an archaeological

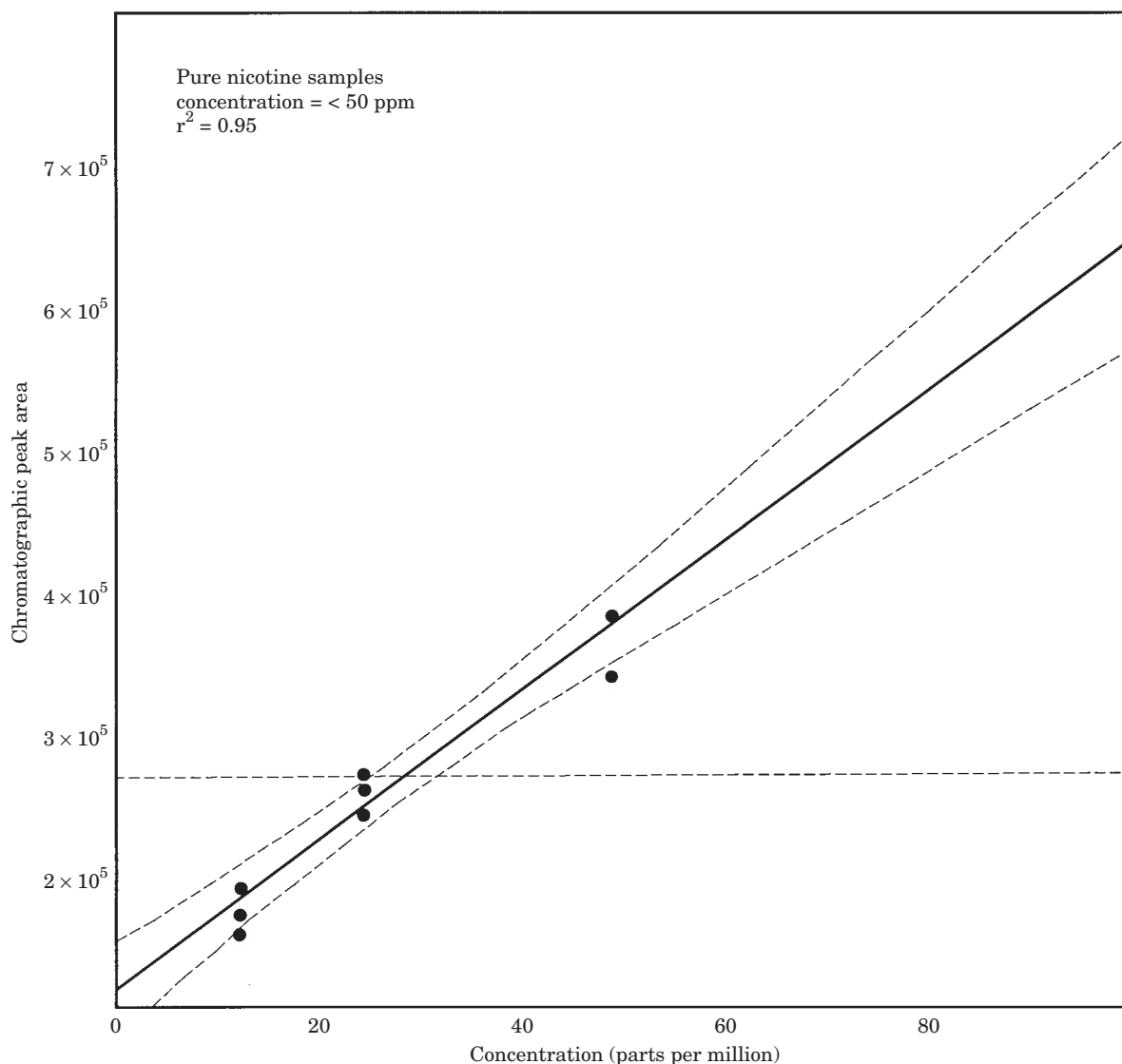


Figure 2.  $r^2$  of GC/MS assays of nicotine/methanol, 10–50 ppm Concentrations.

context (Fletcher, 1979) require experimental verification. The presence of alkaloids in ancient plant tissues has been previously documented (Raffauf & Morris, 1960), so chemical detection should be possible.

Chemical analysis technology has developed since 1922, with advances in spectrographic and chromatographic techniques, and in computer applications to derive chemical identifications. Nicotine has been detected using gas chromatography/mass spectroscopy (GC/MS) in a small sample of smoking pipes from the southwestern United States (Gager, 1991). My own research (Rafferty, 2001) uses GC/MS to investigate the antiquity of tobacco in the Eastern Woodlands of North America. What follows is a technical description of the technique and a summary of the results.

### Detection of Nicotine by Gas Chromatography/Mass Spectroscopy

The project used an analytical protocol adapted from those used by Gager (Gager, 1991; Gager, Johnson & Holmes, 1960) and by Zahlens & Nilsen (1994). The first stage is the extraction of nicotine from the residue. This is done by refluxing an organic solvent, methylene chloride, through a soxhlet extractor which percolates the solvent through the sample in a closed system. The solvent extracts the nicotine as well as other alkaloids that are soluble in this solvent. The sample is then concentrated by evaporating the methylene chloride solvent under nitrogen. This concentrated extract is injected into the GC/MS instrument (Hewlett Packard

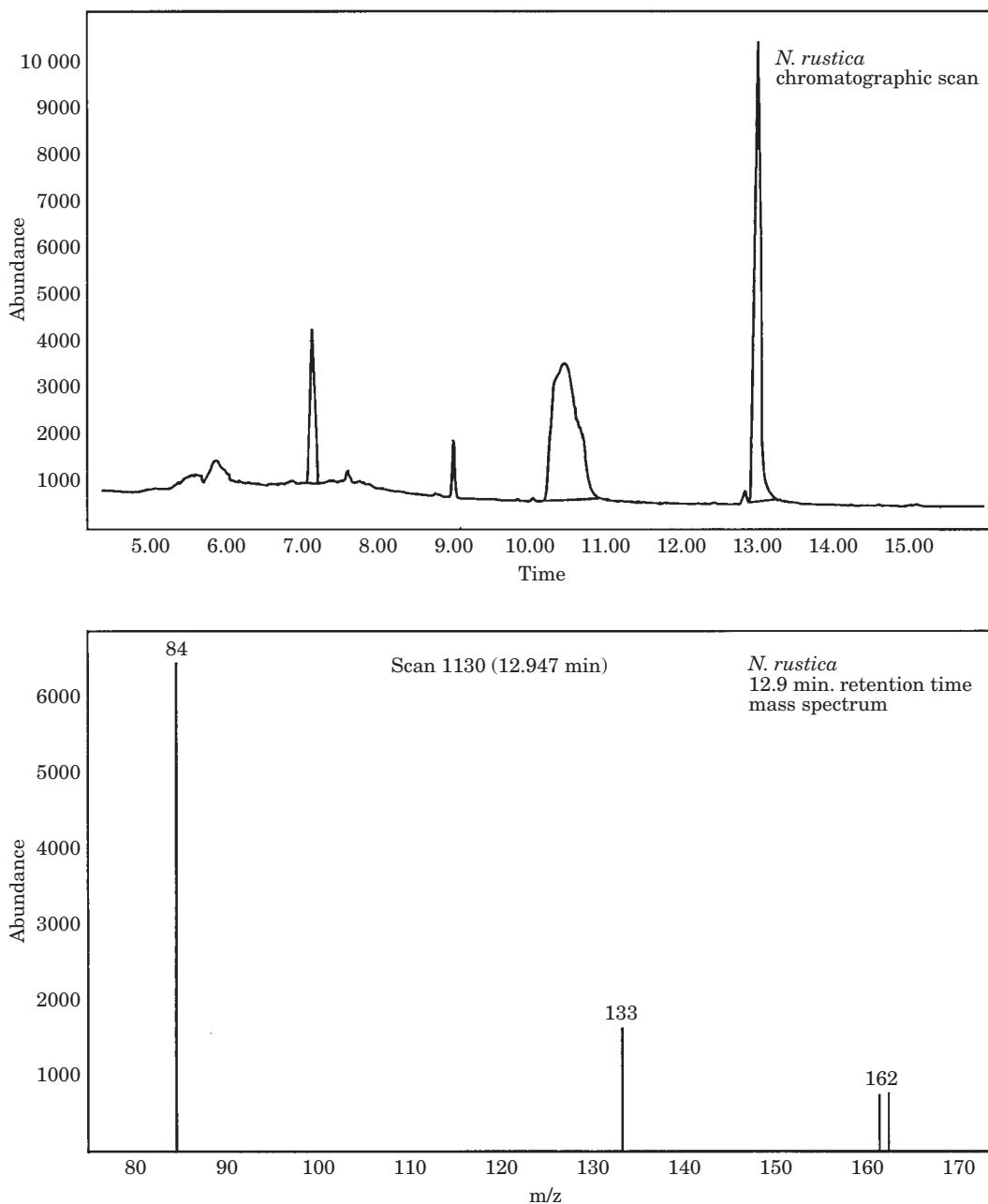


Figure 3. Gas chromatogram/mass spectrum for *Nicotiana rustica*.

5890 GC/MS with Mass Selective Detector with an HP-1 glass capillary column).

The sample is volatilized at the injection port and eluted through a capillary column under increasing temperature. As the sample moves through the column, various components are separated due to their affinity for the stationary phase of the column and can be identified by retention time (the time it takes for a compound to pass through the column and gas chromatograph system). Each chemical component in a sample has a distinct retention time measured in minutes, shown in a peak on a graph which measures

abundance on the ordinate against retention time on the abscissa (see Figure 1). The integrated peak is correlated to the concentration of the chemical. Nicotine has a retention time of 12.9 min, shown in Figure 1, which depicts the chromatogram for pure nicotine. In the nomenclature of the International Union of Pure and Applied Chemistry (IUPAC), Nicotine is termed "Pyridine, 3- (1-methyl-2-pyrrolidinyl)".

A mass selective detector breaks up each chromatographic component into fragment ions, which are shown by their abundance, with each ion represented

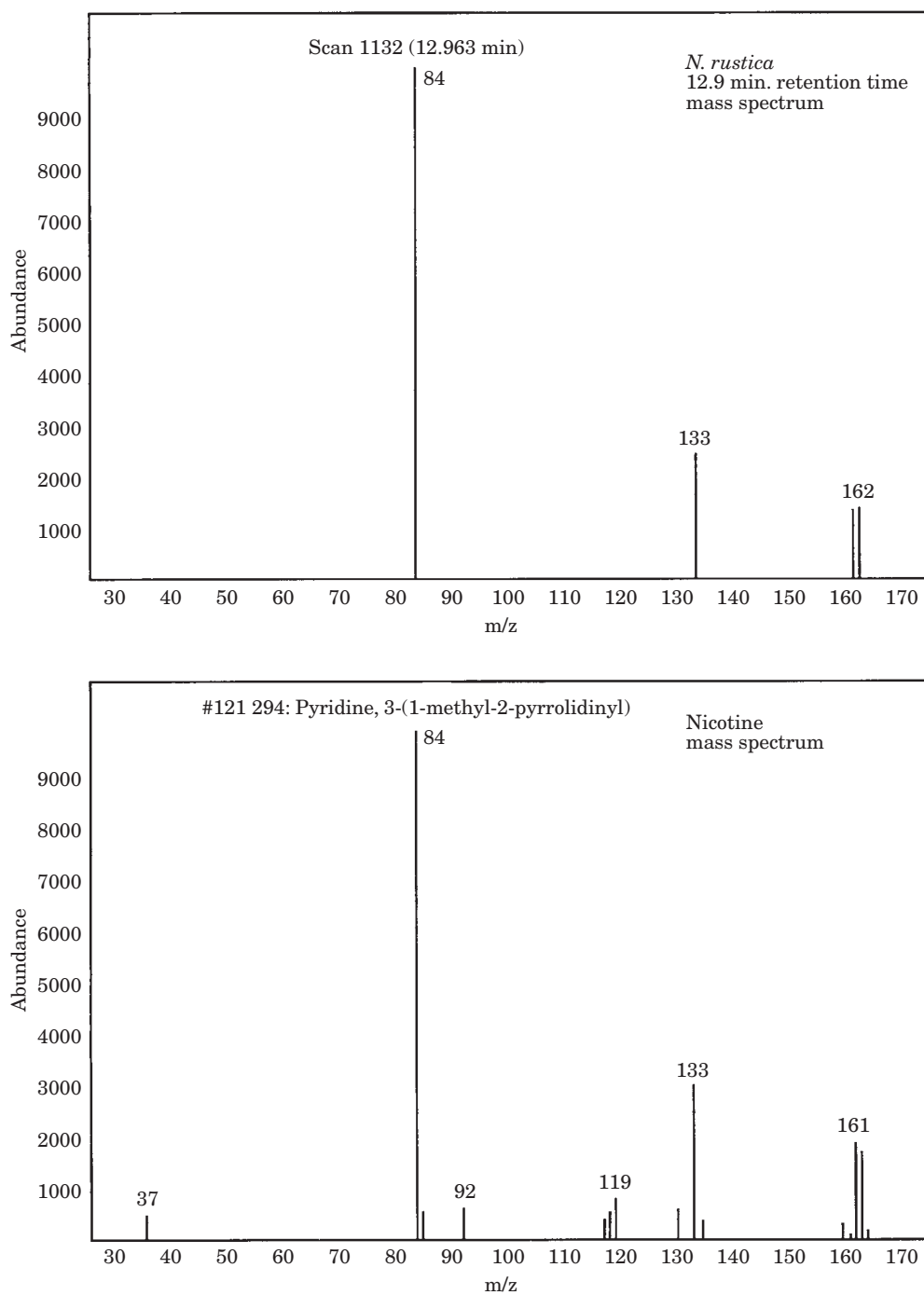


Figure 4. Mass spectrum match for *Nicotiana rustica*.

as a vertical line in increasing molecular weight. The height of each line corresponds to the abundance of that ion. The resulting mass spectrum is unique to that chemical. This mass spectrum forms a "fingerprint" that can identify the compound by a computer search of mass spectra. A computer search of the mass spectra corresponding to all the chromatographic peaks for a sample should yield a statistical match for nicotine at a 12.9 min retention time value if it were present.

Two modes of GC/MS were possible with this instrumental method. First, there is a "Scan" mode which looks at all the constituents of a sample, listing whatever chemical components are present. While Scan mode is inclusive, it is comparatively insensitive. Second, there is a more sensitive "Select Ion Monitoring", or "SIM" mode, which focuses on a specified constituent ion in a target chemical, allowing one to determine its presence or absence. The primary

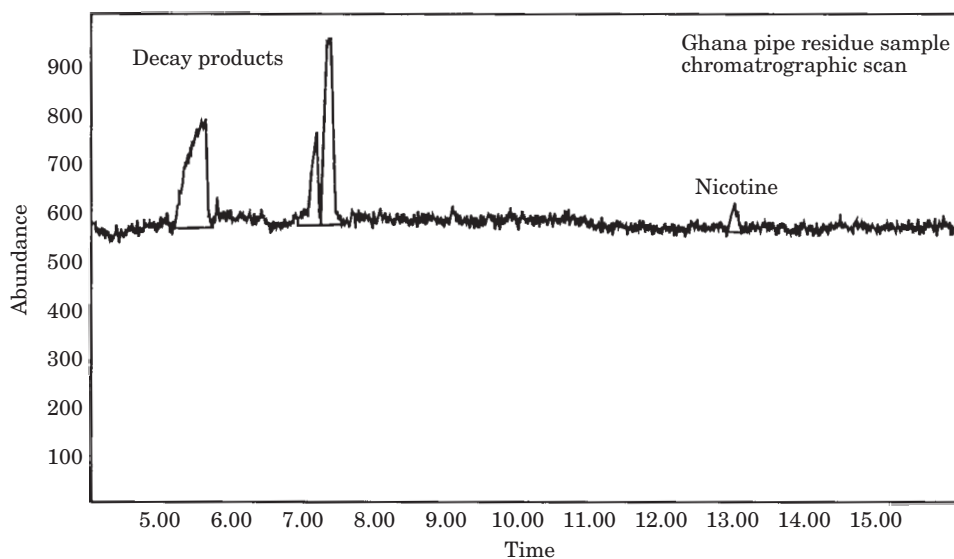


Figure 5. Gas chromatogram/mass spectrum for pipe residue from a 19th-century pipe from Banda Region, Ghana, West Africa.

target ion of nicotine has a mass of 162  $m/z$ . For SIM mode GC/MS you need to know the constituent in advance to test for that specific compound. Since nicotine was present in low concentrations in the extracts, SIM mode was applicable for successful detection.

Before running extractions of archaeological residues it is necessary to ascertain that the GC/MS instrument is operating properly by tuning to an instrumental internal standard, and then running a series of analyses of prepared standards of known concentrations of nicotine. Running a series of nicotine samples also allows the calculation of a correlation coefficient ( $r^2$ ) value to give a value of the precision. Standards of between 10 and 50 ppm were used. The resulting calibration curve is shown in Figure 2, with an  $r^2$  value of 0.95. This indicates a relationship between integrated peak area and sample concentration.

Test extractions were conducted on control samples of modern *Nicotiana rustica*. Figures 3 & 4 show a chromatogram for one sample, along with a computer match for the 12.947 min mass spectrum for nicotine. This illustrated that the procedure extracted nicotine from carbonized tobacco residue if it was present.

The first archaeological sample was from a clay tobacco pipe from the Banda region of Ghana in West Africa dating to the 19th century AD (Stahl, 1994), several hundred years after tobacco had been introduced into West Africa. Figure 5 shows that the chemical signature for nicotine was detected in the Ghanaian pipe, with chromatographic peaks at 5.5, 7.3 and 12.9 min. These are identical figures observed for samples of pure nicotine that had been allowed to break down in solution under laboratory conditions, indicating a characteristic decay pattern of nicotine in related compounds. The 12.9 min peak in the Ghanaian

sample is at the right retention time for nicotine, but is very low in abundance, below the threshold for the computer to identify. However, the 7.3 min peak yielded a positive return for nicotine (Figure 6). The presence of a peak at 12.9 min from a smoking pipe is convincing evidence that nicotine was present on chromatographic criteria, while the 7.3 min peak represents a decay product of lower molecular weight (and therefore a shorter retention time) with a structure similar to its parent compound. The results from Ghana support the efficacy of the technique, and show that nicotine has a “shelf-life” of at least a century, as well as a characteristic decay-signature.

### Early Evidence of Nicotine in Northeastern North America

The site that was selected for GC/MS analysis of early smoking pipe residue was the Cresap Mound Site from West Virginia (Figure 7). The site consisted of a single conical Adena mound containing several burials. The mound was excavated in 1958 under the auspices of the Carnegie Museum of Natural History, and is a primary example of the Adena complex of the central Ohio Valley (Dragoo, 1963). The site contained components from the entire temporal range of the Adena complex, with radiocarbon dates ranging from  $2506 \pm 175$  to  $2020 \pm 150$  BP (with one sigma calibrated ranges of 320 BC to AD 250 using OxCal v.3.3, based on atmospheric data from Stuiver *et al.*, 1998). Three tubular pipes were recovered at Cresap. One was recovered from nearby what is probably a mortuary processing pit at the base of the mound (Feature 30) (Dragoo, 1963: 54). The other two pipes were found in burials, one of a young adult (Burial 26), the other of an adult male (Burial 48). The young adult burial contained

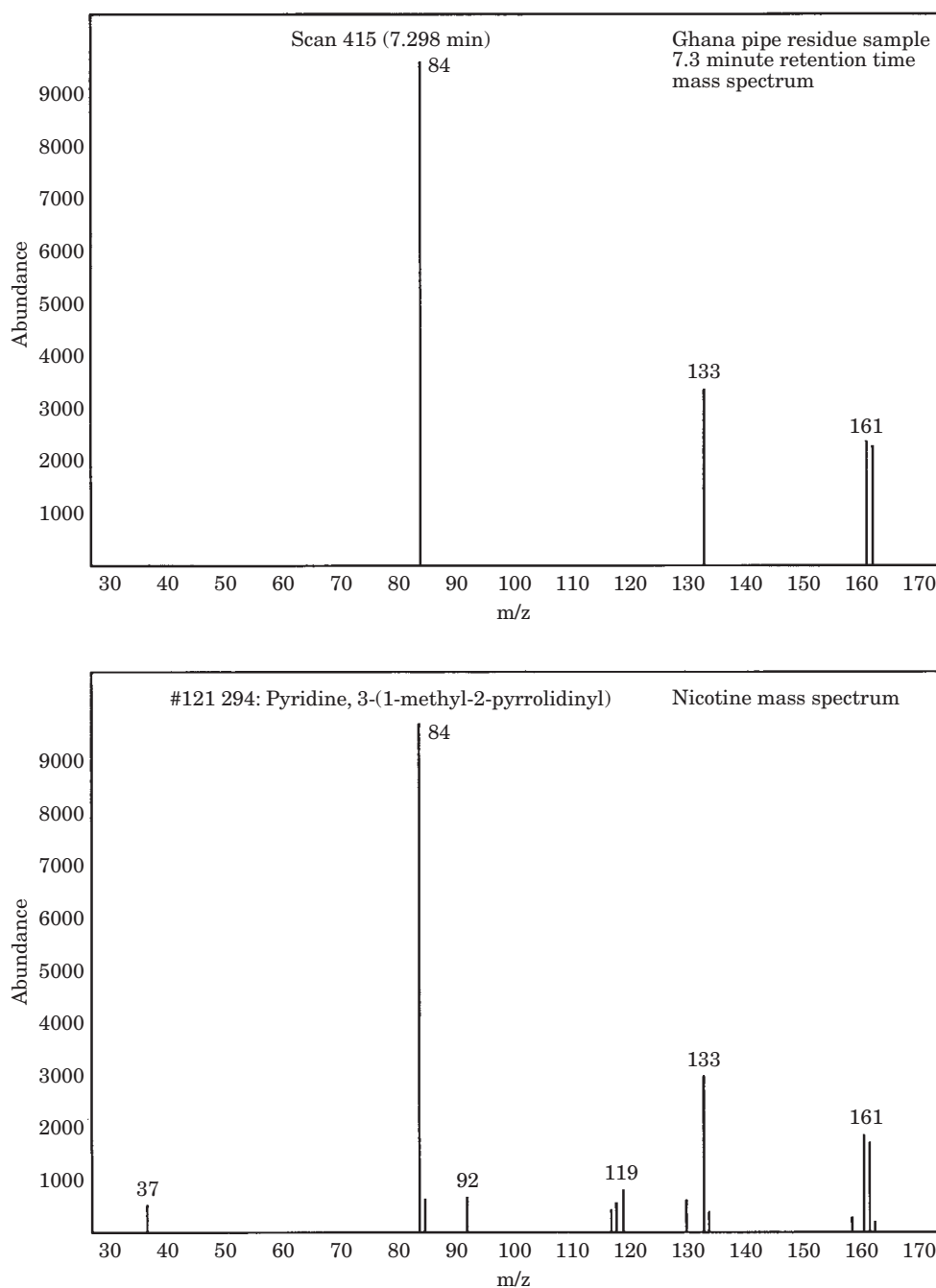


Figure 6. Mass spectrum match for pipe residue from a 19th-century pipe from Banda Region, Ghana, West Africa.

other grave goods, including chipped and ground lithics, a bone tool, copper beads and hematite, while the adult male was interred with only the pipe.

The single pipe associated with Burial 48 had been cremated and then interred with the burial. When burned, the pipe was full of organic material, and a quantity of ash was associated with its remains. The staff of the Carnegie Museum donated a portion of this material for GC/MS analysis. Figures 8 & 9 show the results of the GC/MS assay of the single pipe from

Cresap. Figure 8 shows the gas chromatogram for the Cresap sample. The chromatographic peaks for the sample are similar to the decay signature for nicotine identified from the 19th-century West African sample. The presence of a small peak at a retention time of 12.48 min, and larger peaks at between 5 and 7 min, indicates that the sample was derived from tobacco. One difference is the large peak at approximately 6 min which has so far not been identified. Figure 9 shows the mass spectrum of one specific peak, at a retention time

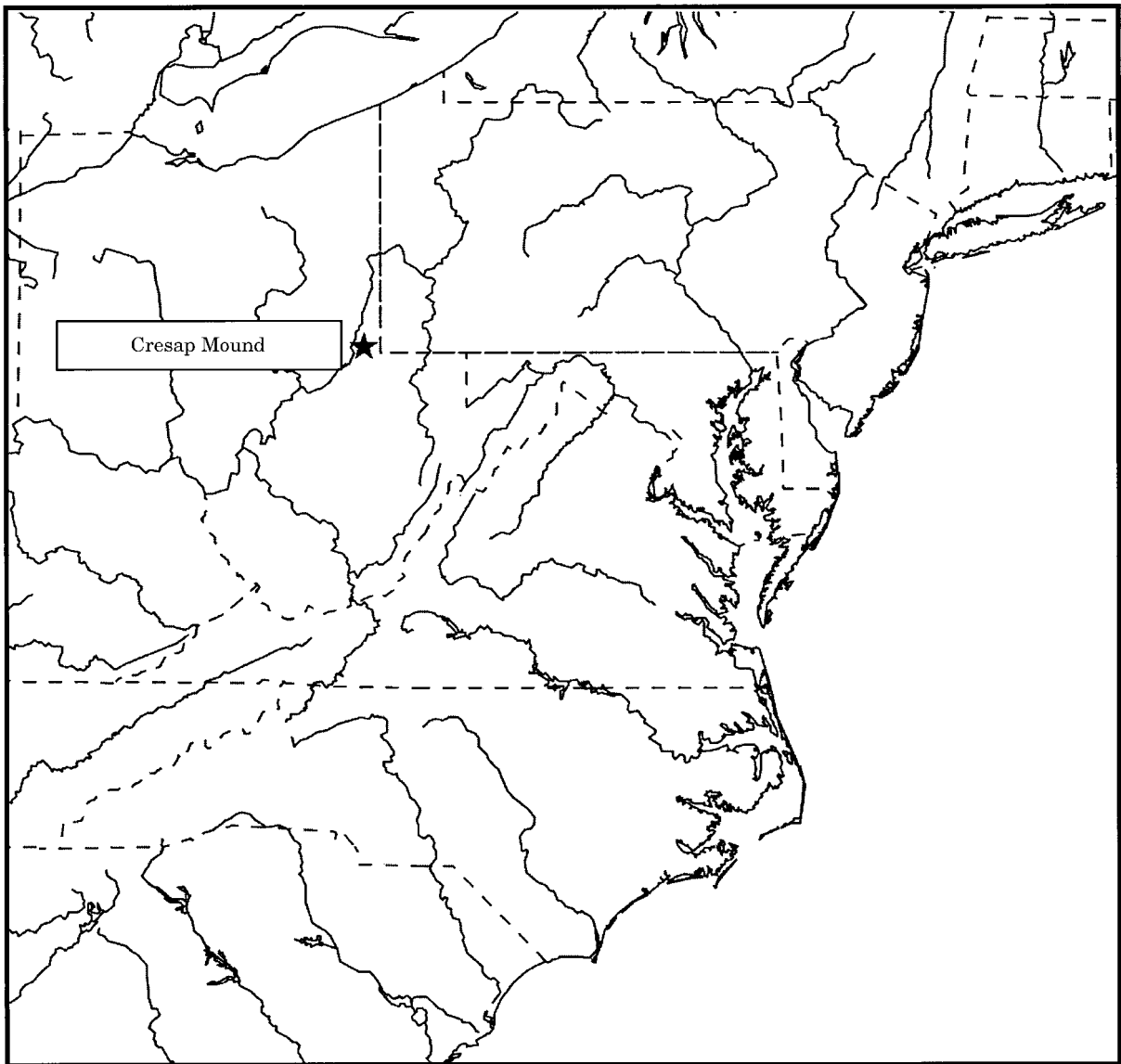


Figure 7. Location of the Cresap Mound Site, West Virginia.

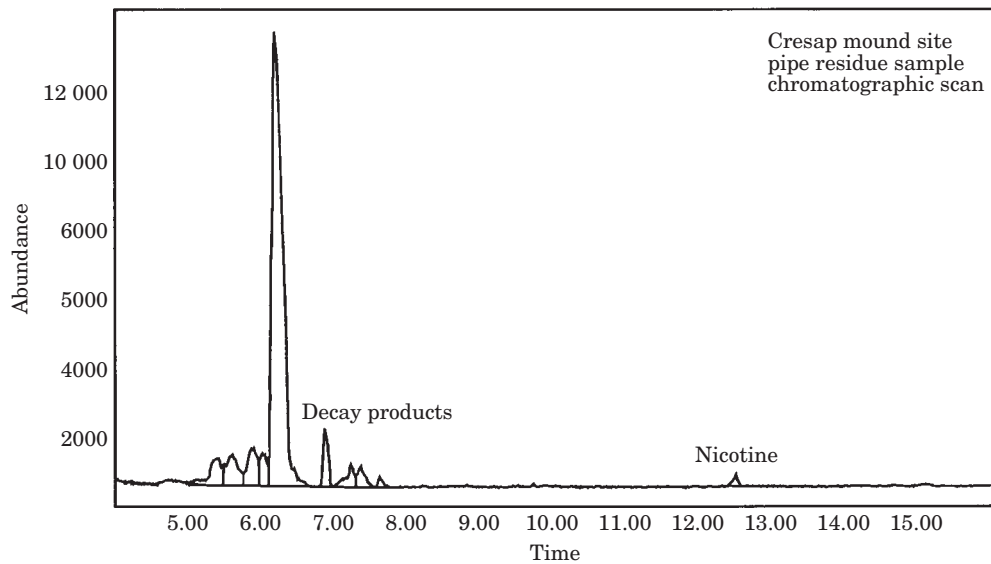


Figure 8. Gas chromatogram for pipe residue from Burial 48, Cresap Mound Site.

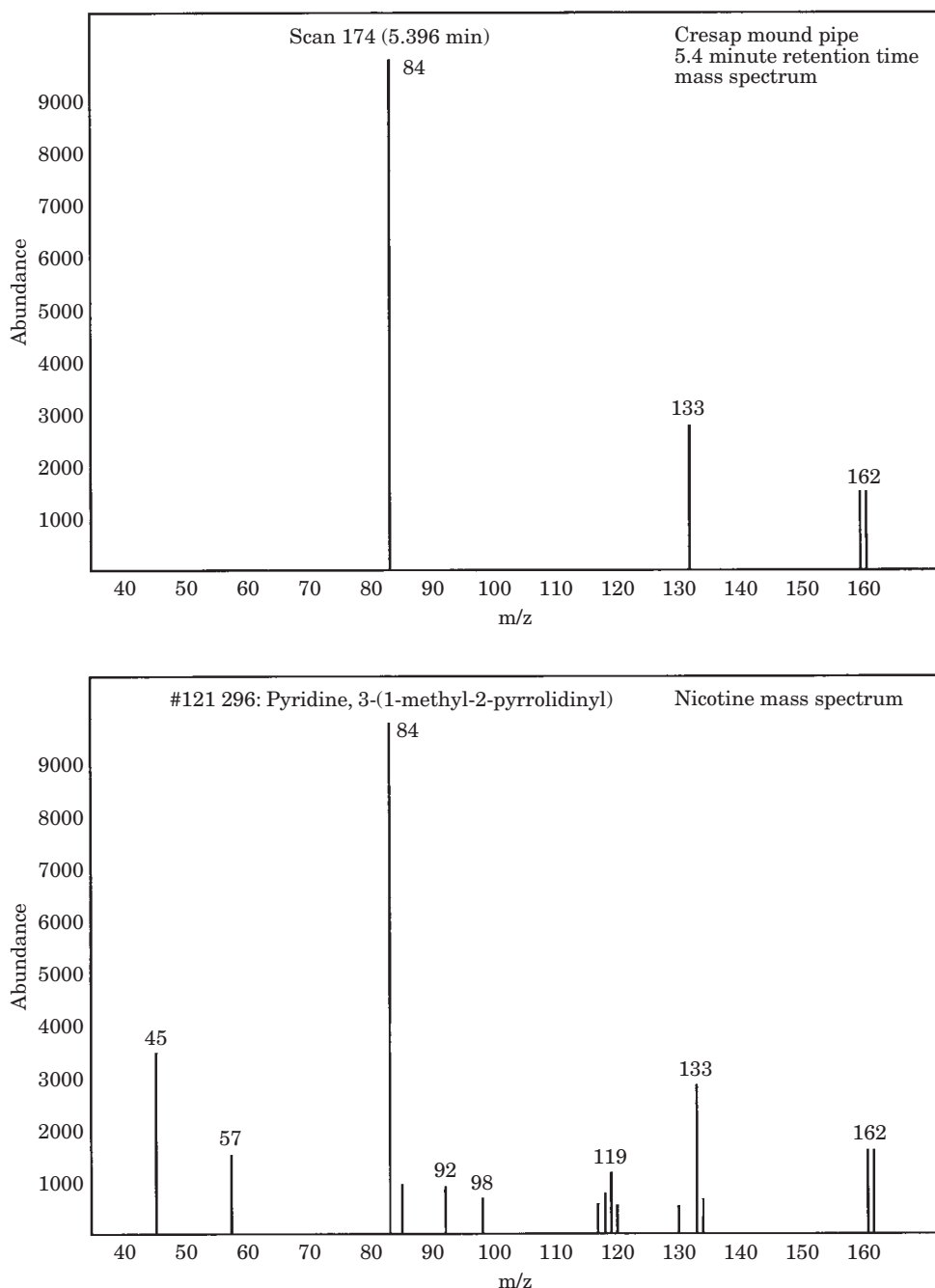


Figure 9. Mass spectrum match for pipe residue from Burial 48, Cresap Mound Site.

of 5.3 min, with a computer match to nicotine, indicating that we have detected a degradation byproduct of nicotine. Peaks at 7.3 and 7.6 min correlated with computer matches for nicotine, also indicating degradation products.

It appears that a plant with a high level of nicotine was smoked in Adena pipes. While Native Americans have been ethnohistorically documented to have smoked numerous plants (Yarnell, 1964), reference to phytochemical literature (e.g., Raffaui, 1970) does not

show any of those particular species has containing nicotine, aside from *Nicotiana* sp. tobacco. If the material smoked in the Cresap Mound pipe was a species of *Nicotiana*, then the location of this pipe near the base of the mound indicates that tobacco dates very early in the Adena phase, possibly prior to 400 BC, as earlier phases of the site date to between 730 and 375 BC (Dragoo, 1963: 290–291). This is in agreement with accepted early dates for Adena, which generally hold the earliest manifestations of the complex to

c. 500 BC (Griffin, 1978). While this is not much earlier than the Middle Woodland evidence cited previously, it is the first evidence pointing to the possibility of tobacco for an Adena site.

However, while other historically smoked plants besides tobacco are not known to contain nicotine, tobacco species are still not the only plants to contain the alkaloid, which is found in a variety of species within and outside of the Solanaceae family. *Asclepias* sp. plants, such as Milkweed or Swallow-Wort, as well as False Daisy (*Eclipta* sp.) are native to eastern North America, and also contain nicotine (Raffauf, 1970). In the absence of macrobotanical evidence, the plant smoked in the Cresap pipe could be any one of several candidates, although tobacco is still the mostly likely possibility. Also, all tobacco species contain some amount of nicotine, with the concentration varying between species (Sisson & Severson, 1990), as well as other alkaloids such as nornicotine and anabasine that are chemically similar to nicotine (Manceau, Fliniaux & Jacquín-Dibreuil, 1992; Saitoh, Noma & Kawashima, 1985; Sisson & Severson, 1990), and might also yield a positive trace for nicotine by this method. It is not possible, therefore, to determine a particular tobacco species from these results, only that it was a plant with a high natural concentration of nicotine or a closely related compound.

## Conclusions

It would appear that the ethnohistorically and ethnographically documented significance of tobacco and of smoking pipes for Native American ceremonial practice has a considerable temporal depth. While these results are equivocal in regards to the specific plant that was used in the Early Woodland pipes, the documentation of nicotine in the pipe residue does indicate that whatever plant was smoked, it was the psychoactive properties of nicotine that were sought by the smoker. Nicotine, and related compounds, ingested as smoke, would have induced altered states of consciousness sought by ritual participants and religious specialists (von Gernet, 2001).

While the application of residue analysis to smoking pipes has proven to be a useful analytical tool, several limiting factors need to be addressed. As mentioned, ethnohistoric evidence shows literally dozens of plants were smoked by Native Americans. To seek evidence for all possible plant candidates by this method would require extensive research to determine a chemical fingerprint for each individual plant, since a target ion must be known in advance. Second, my sample of archaeological residue specimens totals only two, only one of which approaches the earliest known tobacco use from botanical evidence in the Eastern Woodlands. While other samples from Early Woodland contexts have been sought, most of these pipes have lain thoroughly washed in collections for decades, with no

ash residue present. Further research would require recently excavated and unwashed pipes. It is my hope that the presentation of these results will encourage additional chemical research on the early history of smoking.

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