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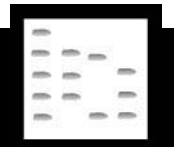
TOBACCO VOLATILES: GAS CHROMATOGRAPHY



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Introduction

The leaves of tobacco plants, *Nicotiana tabacum*, have been shown by Tso and Stedman to consist of a wide array of organic and inorganic compounds. For general classification, the components of the leaf can be described as volatile, semivolatile and nonvolatile. The nature of the compounds making up the components of the leaf range from nonpolar constituents such as hydrocarbons to very polar compounds such as carboxylic acids and amines. The structural identity of these components has been determined in many laboratories, including those of DeMole and Lloyd, through the use of classical wet chemical separation techniques coupled with end determinations involving, in some cases, further separations by gas chromatography (GC). In some cases, headspace volatiles have been trapped on Tenax or charcoal then either thermally or solvent desorbed. Supercritical carbon dioxide has also been used for extraction. In some of the earlier work, the separations were performed on packed glass GC columns and somewhat later on coated glass columns.

With the advent of multidimensional GC (MDGC), the labour-intensive sample preparation techniques associated with wet chemical separations of selected fractions were for the most part eliminated. With the MDGC approach, a concentrated solution of a tobacco essential oil in a volatile organic solvent can be directly injected on to a fused silica capillary precolumn, followed by heart-cutting on to another column. The phase of the pre-column of the MDGC system essentially serves as a replacement for the wet chemical separation approach. In this way, as many as 80 new compounds were tentatively identified in a flue-cured tobacco essential oil. Detection and identification of the volatile components of the essential oils were possible through the use of mass selective detectors and matrix isolation Fourier transform infrared spectroscopy.

Another approach for the lower molecular weight volatiles employed automated purge-and-trap (P & T)-GC with flame ionization (FID) and mass selective detection (MSD). A relatively nonpolar DB-5 ((5% phenyl)methylpolysiloxane) column was employed for the separation of the volatiles. In further advances on the P & T method, the influences of ionic strength and pH on the yield of volatile materials have been reported. By employing a cryofocusing approach coupled with a DB-1701 ((14% cyanopropyl-phenyl)methylpolysiloxane) fused silica column of intermediate polarity, and an internal standard, semiquantitative analytical data were provided on the volatiles from a variety of natural products.

Through the use of multiple fused silica GC columns of different bonded phases, a universal commonality describing the nature of the volatile materials from a wide range of natural products has been developed. The types and profiles of volatile low molecular weight compounds found in the headspace above tobacco leaves has been shown to bear remarkable similarities to the profiles from a diverse array of heat-treated natural products such as coffee, peanuts and soybeans. The common thread linking all of the products has been ascribed to the presence of specific reactions between amino acids and other nitrogen sources with sugar molecules to yield volatile components such as pyrazines and aldehydes. For details of the work outlined in this section, see the papers by Coleman *et al.* in the Further Reading section.

This article describes the current GC approaches to the analysis of volatiles from tobacco. Results are described on approaches involving dynamic headspace-GC-MSD, as well as automated solid-phase microextraction (SPME)-GC-MSD.

Experimental

Dynamic Headspace P & T-GC-FID-MSD

Solid samples Representative samples of dry cured tobacco leaves were finely ground in a coffee grinder prior to analysis. After grinding, the moisture content of the tobacco leaves was adjusted to 20% by weight and the samples were allowed to age overnight at room temperature ($\sim$23°C). Finely ground moisture-adjusted tobacco leaves (3–5 g) were placed in a 25 mL sampling tube and affixed to the sampling port of a Tekmar LSC 2016/LSC 2000 P & T unit. Six replicates w ere run to obtain adequate statistical analyses. Average % relative standard deviation values for the total FID area counts for the solid samples were approximately $\pm$10%. After reaching the desired temperature, the contents of the 25 mL sampling tube were swept with helium, at a known flow rate for a specified length of time. The volatiles in the gas stream were efficiently trapped on a Tenax column (12 in $\times$1/8 in i.d. SS tube) held within the Tekmar LSC 2000 unit. After sweeping the sample, the contents of the Tenax trap were thermally desorbed and transferred via a heated, aluminium-clad, deactivated, 0.53 mm i.d. fused silica transfer line to the injection port of a Hewlett Packard (HP) 5880 GC set at 250°C. Liquid nitrogen was employed to cool the GC oven to 0°C during the transfer of the volatiles. Upon completion of the transfer, the GC oven was temperature-programmed up to 250°C. The effluent from the DB-1701 column was split via an SGE low dead-volume splitter to the FID and MSD detectors at a ratio of 20 : 80. An HP 5970 Mass Selective Detector operation at 70 eV in the electron impact mode served as the MSD. The analyses were automated by means of basic programming of the HP 5880 GC terminal. The parameters employed in the analysis of the volatiles from solid tobacco leaves are listed in **Table 1**.

The volatile constituents were identified with the aid of GC retention indices as well as results of library mass spectral searches of Wiley, NBS and internal mass spectral databases.

Aqueous liquids and suspensions To a 5 mL sparge tube was added 1 mL of the tobacco liquid extract or suspension. The sparge tube was affixed to the Tekmar LSC 2016. Analysis then proceeded as described in the preceding section.

Table 1 Instrumental parameters for P & T-GC-MSD-FID analyses

Sample volatilization temperature	70°C
Sample volatilization time	20 min
Trapping material	Tenax
Trap desorption temperature	175°C
Trap desorption time	10 min
GC oven initial temperature	0°C
GC oven first programme rate	2°C min^{-1}
GC oven final value	47°C
GC oven second programme rate	10°C min^{-1}
GC oven final value	250°C
GC oven final time	10 min
GC column	DB-1701
GC column length	30 m
GC column i.d.	0.32 mm
GC column film thickness	1 μm

Automated SPME-GC-MSD

Aqueous liquids and suspensions A Varian 8200 CX AutoSampler with SPME II Sample Agitation was mounted on top of an HP 5890 Series II Plus GC equipped with a HP 5972 MSD operating either in the electron impact mode at 70 eV or in the selected ion monitoring (SIM) mode. This GC was fitted with a relatively polar DB-Wax (polyethylene glycol (PEG)) fused silica column (30 m $\times$ 0.25 mm i.d., 0.5 μm film thickness, J & W Scientific). The MSD interface and GC injection port temperatures were 250°C. The GC oven was temperature-programmed from 40 to 140°C at 5°C min^{-1}, then to 220°C at 10°C min^{-1} and held there for 4 min. Splitless injections were made and the split was opened after 1 min. The fibre was automatically submerged in the solution, vibrated for 0.75 min, removed, injected, and held in the injection port for 30 min, employing parameters set via operating software.

SPME fibres for automated injections were obtained from Supelco and employed strictly following the manufacturer's instructions for use and activation.

Prior to analysis by automated SPME, the aqueous heat-treated tobacco suspensions were manually filtered through a Whatman Autovial equipped with a 0.45 μm polyvinylidenefluoride filter and designed for use with aqueous solutions. Then, to a 1.8 mL vial equipped with Teflon-lined septum, was added, via a Rainin EDP Plus Motorized Microliter Pipet, 1.7 mL of the filtered solution. Strict attention to consistency in the addition of 1.7 mL was necessary to obtain reproducible results. The charged vials were loaded on the sample carousel and automatically sampled employing the instrumentation software provided by Varian and HP. In some cases it was necessary to dilute the heat-treated suspensions to obtain reproducible

fibre performance. Fresh samples were used for every injection.

SIM was used for the quantitative determination of selected pyrazines in the heat-treated suspensions. The selected compounds and accompanying selected ions (m/z) are listed as follows: methylpyrazine, 94, 95, 96; C2 pyrazines, 107, 108, 109, 110; C3 pyrazine, 121, 122, 123, 124 and C4 pyrazines, 135, 136, 137 and 150. The C2, C3 and C4 notations are used to denote a class of pyrazines. For example, C2 pyrazines would include all of the dimethylpyrazines as well as ethylpyrazine. Identical arguments are used for the C3 and C4 terms.

Discussion of Results

The distinct aromas associated with such materials as cured tobacco, roasted peanuts, roasted coffee and baked bread are familiar to most people. The identity of a large majority of these volatile aromatic components has been published recently. Within the tobacco industry, skilled individuals can easily distinguish between the types of tobacco employed in American blended cigarettes. This is not surprising in light of the unique patterns of the headspace volatiles found for these tobaccos (**Figure 1**). In a number of cases, some of the volatiles found in the headspace above the tobaccos are common to all of them and the main difference lies in the relative amounts of these common volatiles. For example, 2-butanone was common to three tobacco samples; however, the relative amounts were significantly different. In 1996 Coleman *et al.* demonstrated that this quantitative difference in the amount of specific headspace volatiles is common to a diverse array of natural products.

Use of the DB-1701 column with intermediate polarity served very well in providing sufficient resolution to assist in identifying the vast majority of compounds. Some of these compounds are listed in **Table 2** with numbers which correspond to peak identities throughout the entire manuscript. Each of the components listed in Table 2 has been previously identified by Tso and Stedman in tobaccos. Thus, P & T-GC-MSD is a viable approach for the identification and segregation of tobacco types.

Roasting of natural products has often been associated with the production of pleasant aromas and tastes. In some cases these pleasant sensory responses have been attributed to the presence of low concentrations of volatile pyrazines and aldehydes. The presence of these compounds has been linked to the reaction of nitrogen sources such as amino acids with carbon sources such as sugars. Toasting of burley tobacco leaves at approximately 150°C for 5 min produces an aroma associated with toasted natural products. Analysis of the headspace above the toasted burley tobacco reveals the presence of low molecular weight compounds also found in other heated natural products (**Figure 2**).

Increased levels of the Strecker aldehydes, 2-methylpropanal, 3-methylbutanal and 2-methylbutanal, relative to the starting tobacco are leading indicators of the sugar–nitrogen reactions. The reaction to form Strecker aldehydes is one of a series of complex reactions, collectively referred to as the Maillard reaction, occurring simultaneously between nitrogen sources such as amino acids and sugars during the heat treatment of natural products. When valine, leucine, isoleucine, phenylglycine and phenylalanine react with, for example, fructose at elevated temperatures, the following Strecker aldehydes are produced: 2-methylpropanal; 3-methylbutanal; 2-methylbutanal; benzaldehyde; and benzeneacetaldehyde. Thus, the presence of these compounds in the headspace above heat-treated natural products serves as an excellent indicator of amino acid–sugar reactions. In addition, an increased amount of volatile pyrazines in the headspace above heated natural products serves as a witness to the reaction between nitrogen sources and sugars. Further changes can be noted in the profile of the toasted burley relative to the starting material. Substantial increases in the relative amounts of solanone, neophytadiene and damascenone, all known burley constituents, can be found in the toasted material (**Figure 3**).

As before, the intermediate polarity of the DB-1701 column served well in providing sufficient chromatographic resolution of the volatile components.

Cooking natural products to produce edible materials with positive sensory attributes in some instances occurs in what can be viewed as an aqueous suspension. Boiling rice, beans or cooking a roast under pressure are simple examples. It has been shown that heat treatment of an aqueous suspension of burley tobacco, at 170°C for 30 min, produces some similar volatile materials (**Figure 4**) as are found in the headspace, for example, above a heat-treated peanut suspension. The headspace of both samples is dominated by the presence of Strecker aldehydes, sugar thermal degradation products and pyrazines. On a relative basis, the volatile materials detected in the heat-treated suspension were not detectable in the starting tobacco. The volatile components in the heat-treated aqueous burley tobacco suspension were also indicative of the sugar–nitrogen chemistries known to produce Strecker aldehydes and volatile pyrazines when heating tobacco alone.

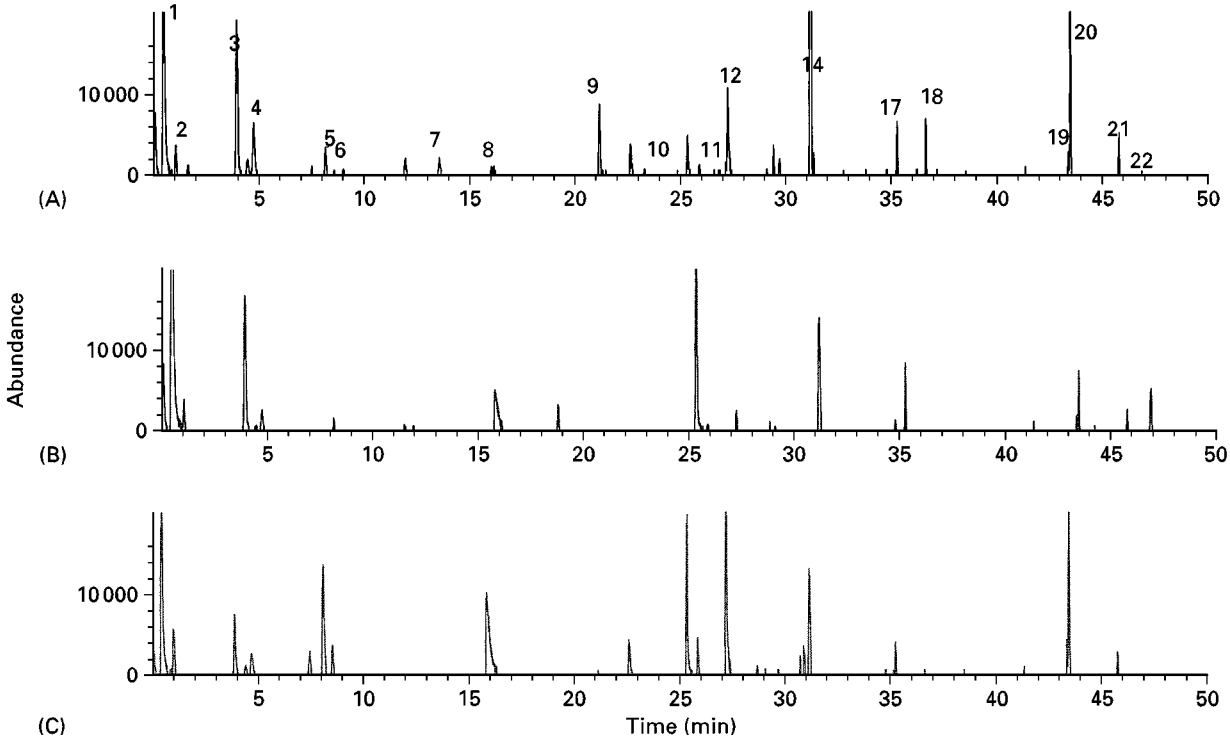

Figure 1 Headspace volatiles from dynamic headspace analysis of selected tobaccos. (A) Turkish; (B) flue-cured; (C) burley.

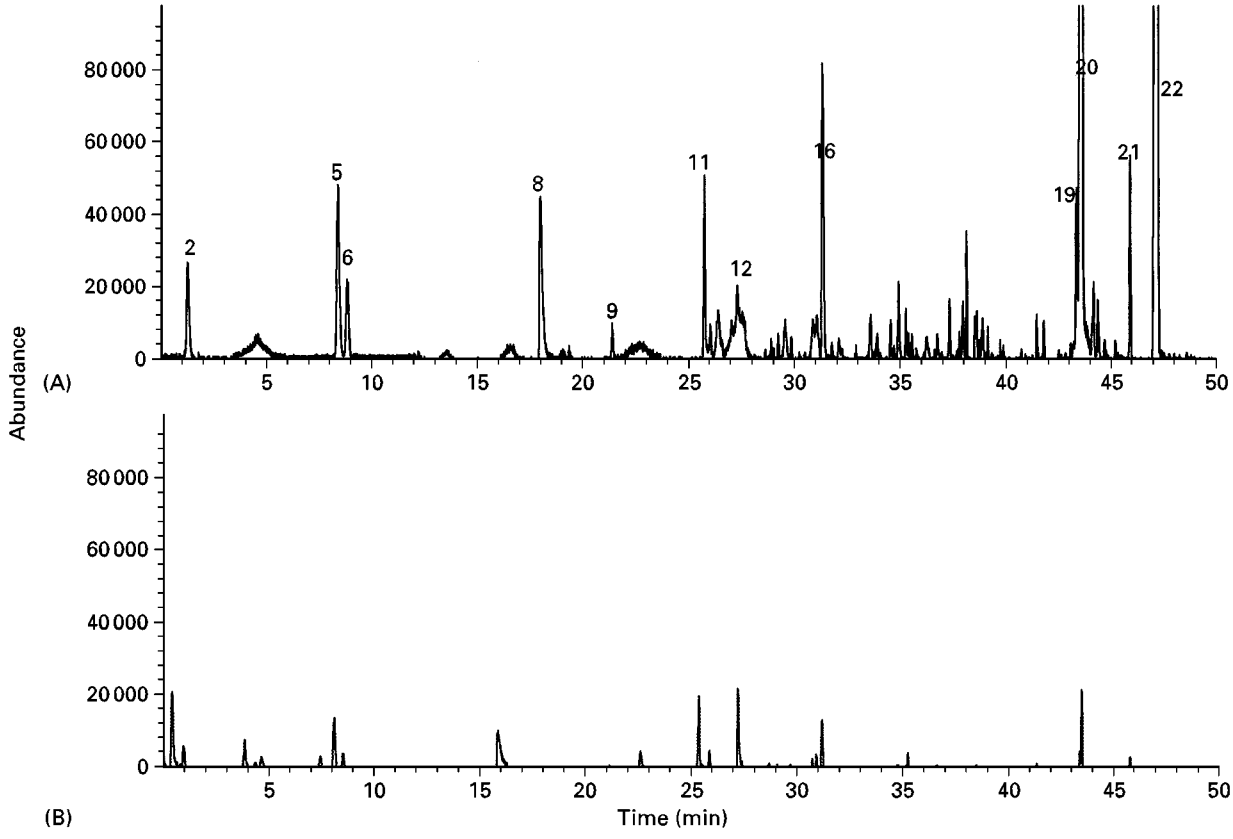

Figure 2 Headspace volatiles from dynamic headspace analysis of (A) toasted burley and (B) burley tobacco.

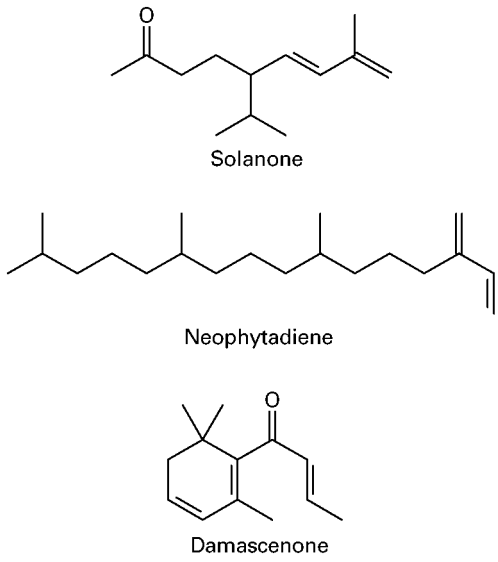

Figure 3 Structures of some compounds present in heat-treated tobaccos.

Certain amino acids have been postulated in model systems by Waller and Feather to be directly involved in the production of pyrazines and selected aldehydes by means of the Strecker degradation mechanism. That is, upon reaction with selected sugars, certain

Table 2 Compounds identified and numbered in selected figures

Compound number	*Identification*
1	Acetone
2	2-Methylpropanal
3	2-Butanone
4	2,3-Butanedione
5	3-Methylbutanal
6	2-Methylbutanal
7	2,3-Pentanedione
8	Acetic acid
9	Hexanal
10	Methylpyrazine
11	Furfural
12	C2 Pyrazines[a]
13	Acetylfuran
14	6-Methyl-5-hepten-2-one
15	C3 Pyrazines[b]
16	Benzaldehyde
17	C14 Hydrocarbon
18	C15 Hydrocarbon
19	Nicotine
20	Solanone
21	β-Damascenone
22	Neophytadiene

[a]Pyrazines such as dimethylpyrazine and ethylpyrazine.
[b]Pyrazines such as methylethyl pyrazines.

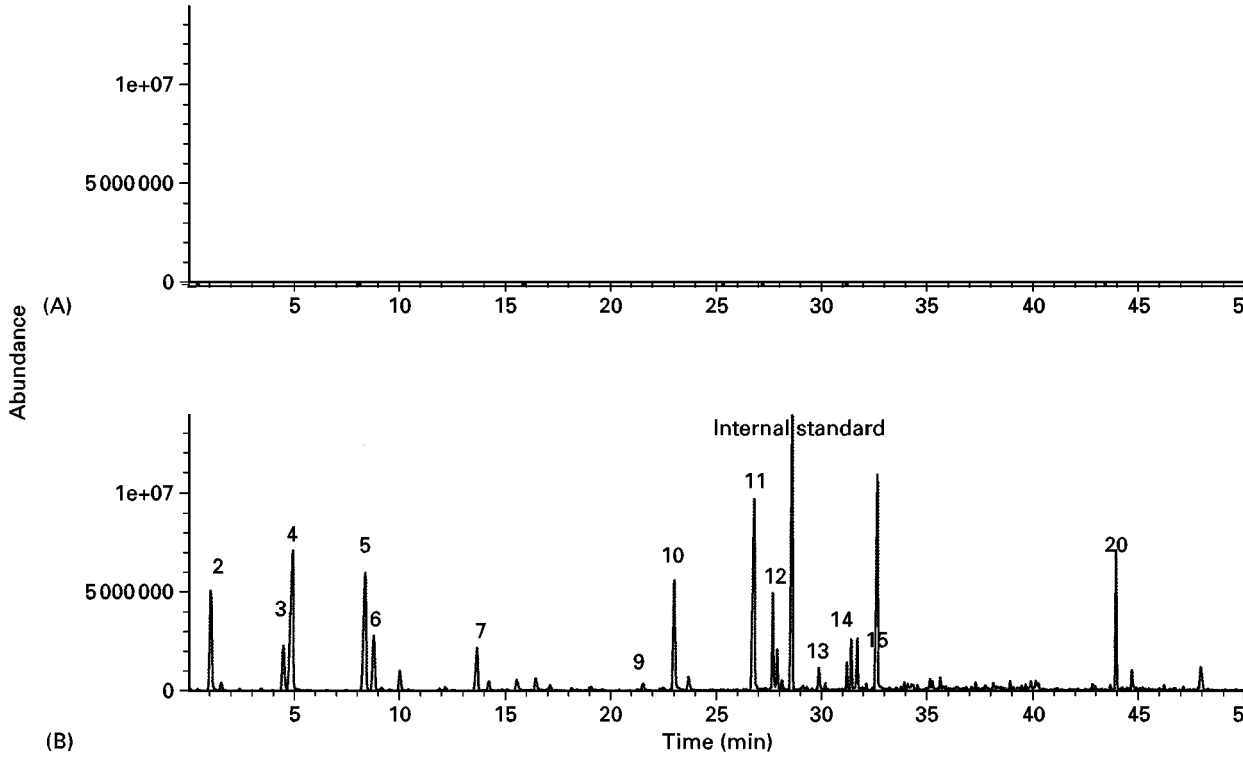

Figure 4 Headspace volatiles from dynamic headspace analysis of (A) burley tobacco and (B) a heat-treated burley tobacco suspension.

amino acids will yield volatile aldehydes corresponding in large part to the structure of the parent amino acid backbone. Specifically, for example, phenylalanine upon reaction with sugars yields as a volatile aldehyde benzaldehyde, while valine will yield 2-methylpropanal. Dynamic headspace analysis of aqueous suspensions of burley tobacco with mmole quantities of added phenylalanine and valine show elevated levels of benzaldehyde and 2-methylpropanal (**Figures 5** and **6**) respectively. These results provide the first evidence implicating the Maillard and Strecker reactions within a tobacco matrix. Again, the DB-1701 GC column provides the necessary resolution.

Recently, manual SPME-GC-MSD has been shown to be an excellent analytical approach for the quantitative determination of volatile components associated with heat-treated natural products. Specifically, the quantitative analysis of pyrazines, furfurals, thiazoles and pyridines has been demonstrated in aqueous media. With the introduction of an automated injection system analytical precision has improved. Application of automated SPME (AutoSPME)-GC-MSD to the analysis of a heat-treated tobacco suspension revealed some of the first insights into a variety of reaction mechanisms occurring during the heating process (**Figure 7**). The origin of pyridine, myosmine, β-nicotyrine and pyrrole-2-carboxaldehyde can probably be directly linked to the decomposition of the alkaloid, nicotine. The origin of the pyrazines is no doubt related to sugar–nitrogen reactions (see above), and the furans can be attributed to the thermal decomposition of sugars. Searches for the appropriate SPME fibre resulted in the selection of a carboxenpolydimethylsiloxane fibre. This particular fibre is suited to the analysis of relatively polar compounds in aqueous solutions. The best column for the analysis was found to be a relatively polar DB-Wax, which provided the most information concerning the reaction mechanisms.

Further application of AutoSPME-GC-MSD in the SIM mode has provided additional insights into the formation of volatile pyrazines during the heat treatment of aqueous tobacco suspensions. Model studies indicate the formation of pyrazines through the coupling of two molecules (**Figure 8**). Thus, should the source of the N in the two molecules be ^{15}N, then the possibility of changes in the m/z values for pyrazines could be either one or two. For example, the m/z for methylpyrazine could be changes from

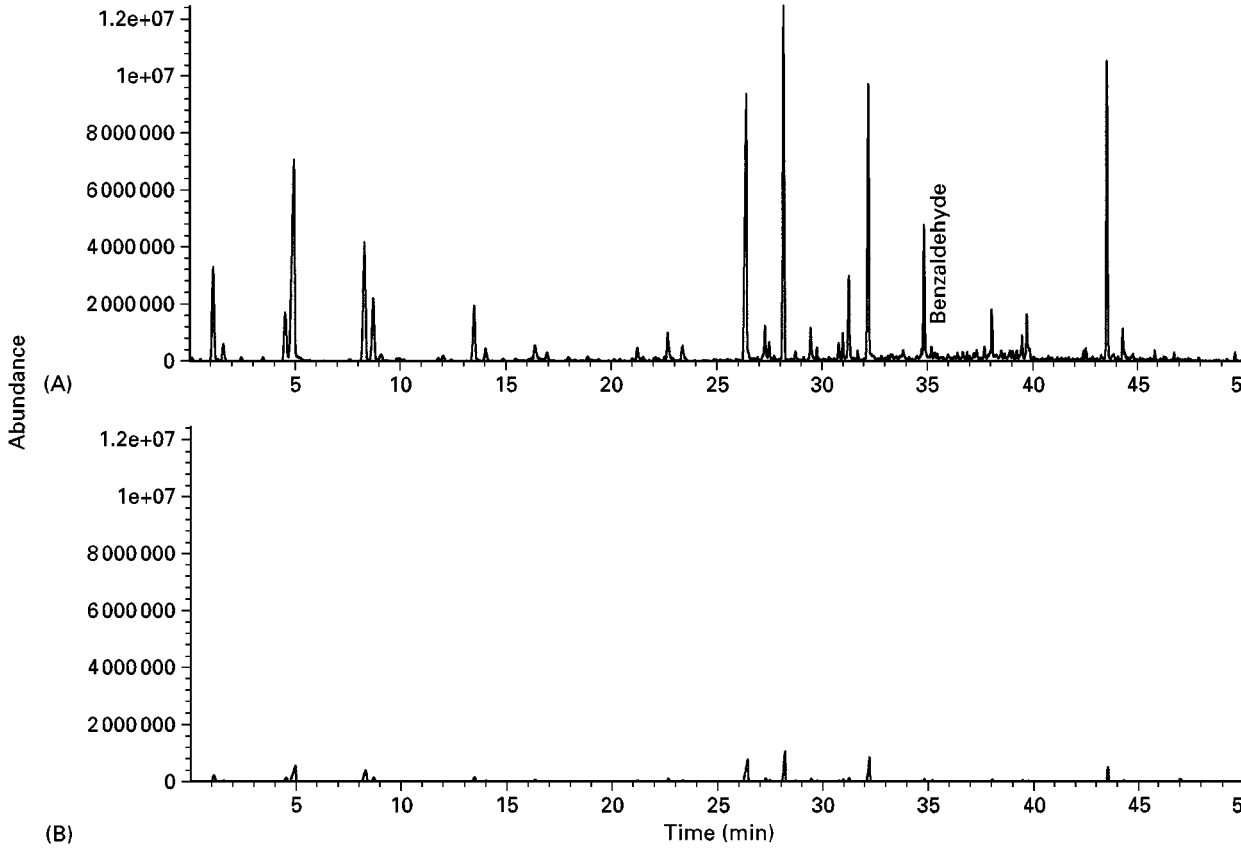

Figure 5 Headspace volatiles from dynamic headspace analysis of two tobacco dusts: (A) tobacco dust plus phenylalanine; (B) tobacco dust.

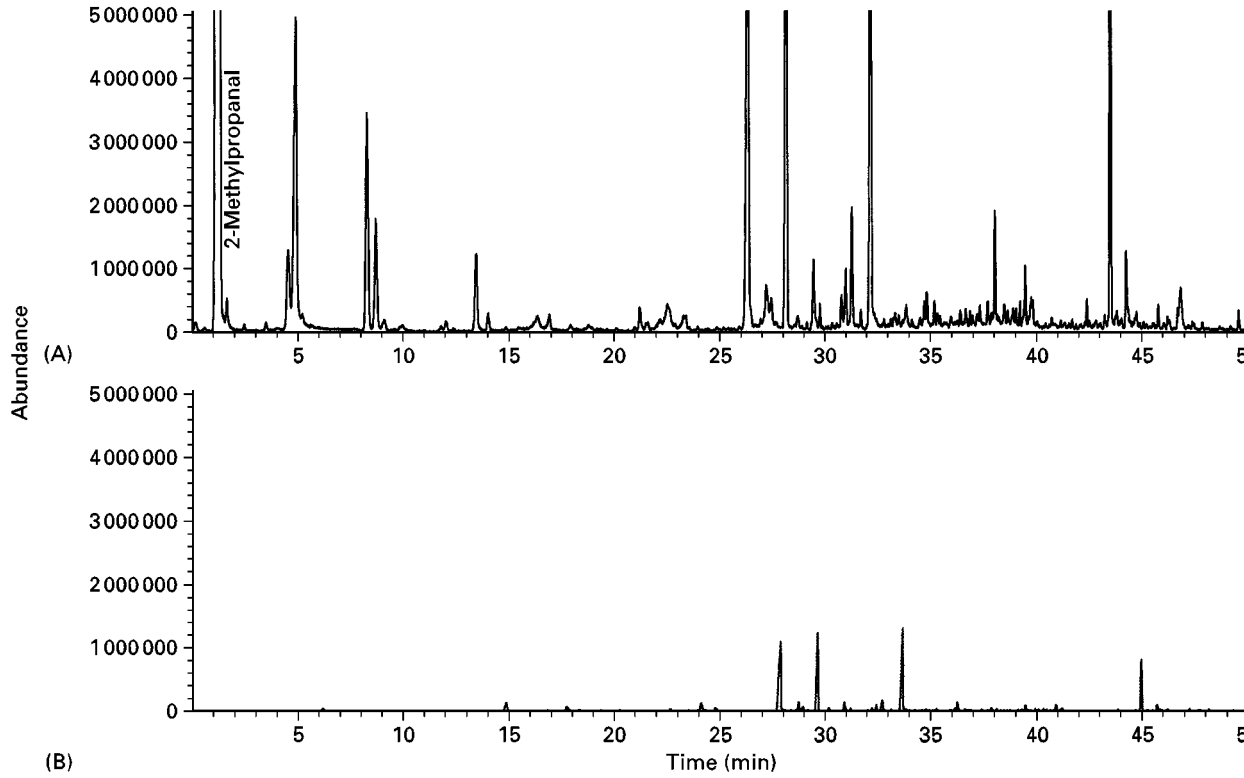

Figure 6 Headspace volatiles from dynamic headspace analysis of tobacco dust suspensions. (A) Tobacco dust plus valine; (B) tobacco dust.

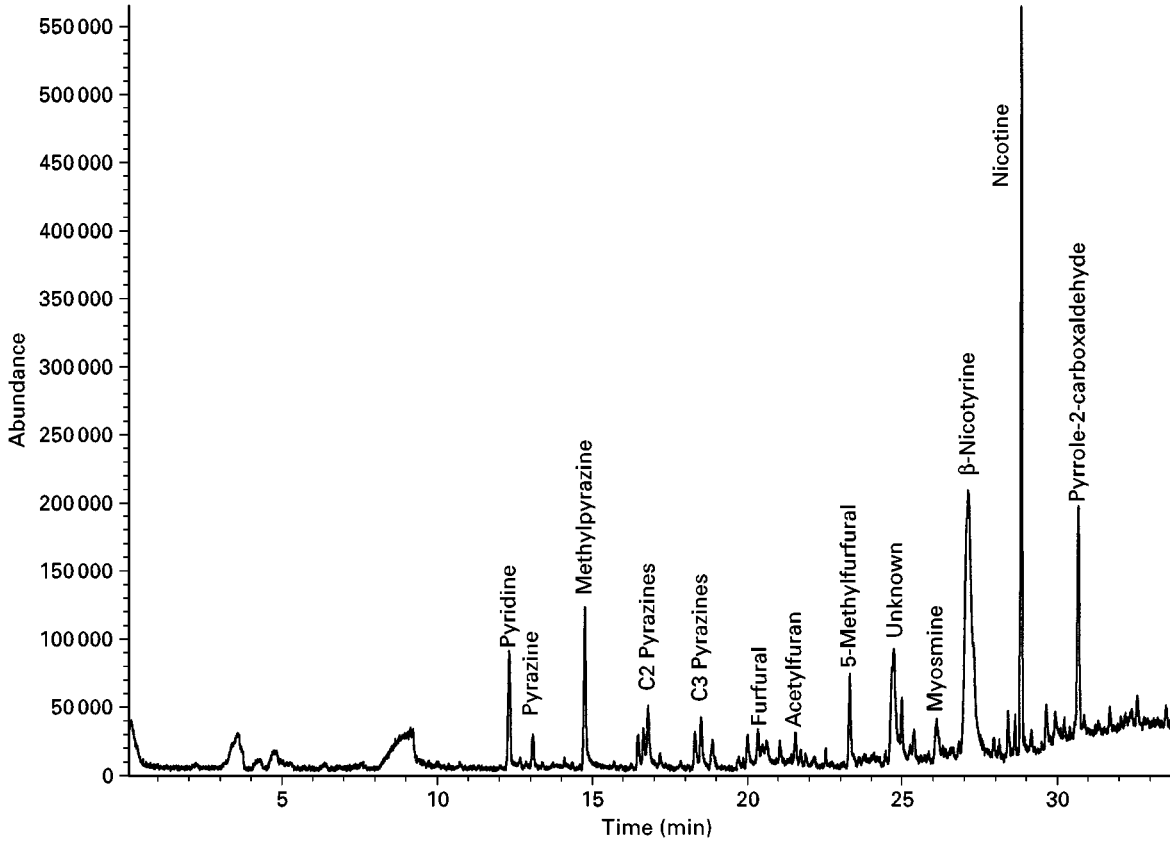

Figure 7 Total ion chromatogram from AutoSPME of aqueous heat-treated tobacco suspension using a carboxen polydimethylsiloxane SPME fibre.

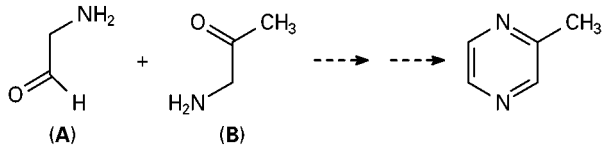

Figure 8 Formation of methylpyrazine from two molecules.

a molecular ion of 94 to molecular ions of either 95 or 96. In other words, the distribution of the ions m/z = 94, 95, 96, would substantially change if ^{15}N were incorporated into the methylpyrazine molecule. Model studies have also indicated that a possible source of nitrogen for the formation of pyrazines is the ammonium ion. Labelling the ammonium ion with ^{15}N would seem to be a viable approach for determining one of the possible mechanisms associated with pyrazine formation in a heat-treated tobacco matrix.

Thus, to an aqueous tobacco dust suspension was added a mmole quantity of $^{15}NH_4OAc$. A control reaction was also performed with no additive. After heat treatment at 170°C for 30 min in a sealed reactor, AutoSPME-GC-MSD-SIM was performed on the filtrate (**Figure 9**), with the focus on methylpyrazine. Alteration of the m/z pattern observed for the methylpyrazine produced in the control experiment was definitely evident. The shift in abundance toward the ions m/z = 95 and 96 directly implicated the inclusion of both one and two ^{15}N atoms into the methylpyrazine molecule, obviously from the added $^{15}NH_4OAc$. Thus, for the first time, by employing AutoSPME-GC-MSD-SIM, a direct link between the presence of naturally occurring reagents such as ammonium ions and the production of volatile aromatic compounds such as pyrazines has been clearly demonstrated.

Conclusion

GC in combination with such sample preparation techniques as automated dynamic headspace and SPME has been demonstrated to be an effective analytical tool for the qualitative and quantitative examination of tobacco volatiles. Through the use of an MS detector in the SIM mode, understanding has been acquired of the formation of the volatiles on a molecular basis.

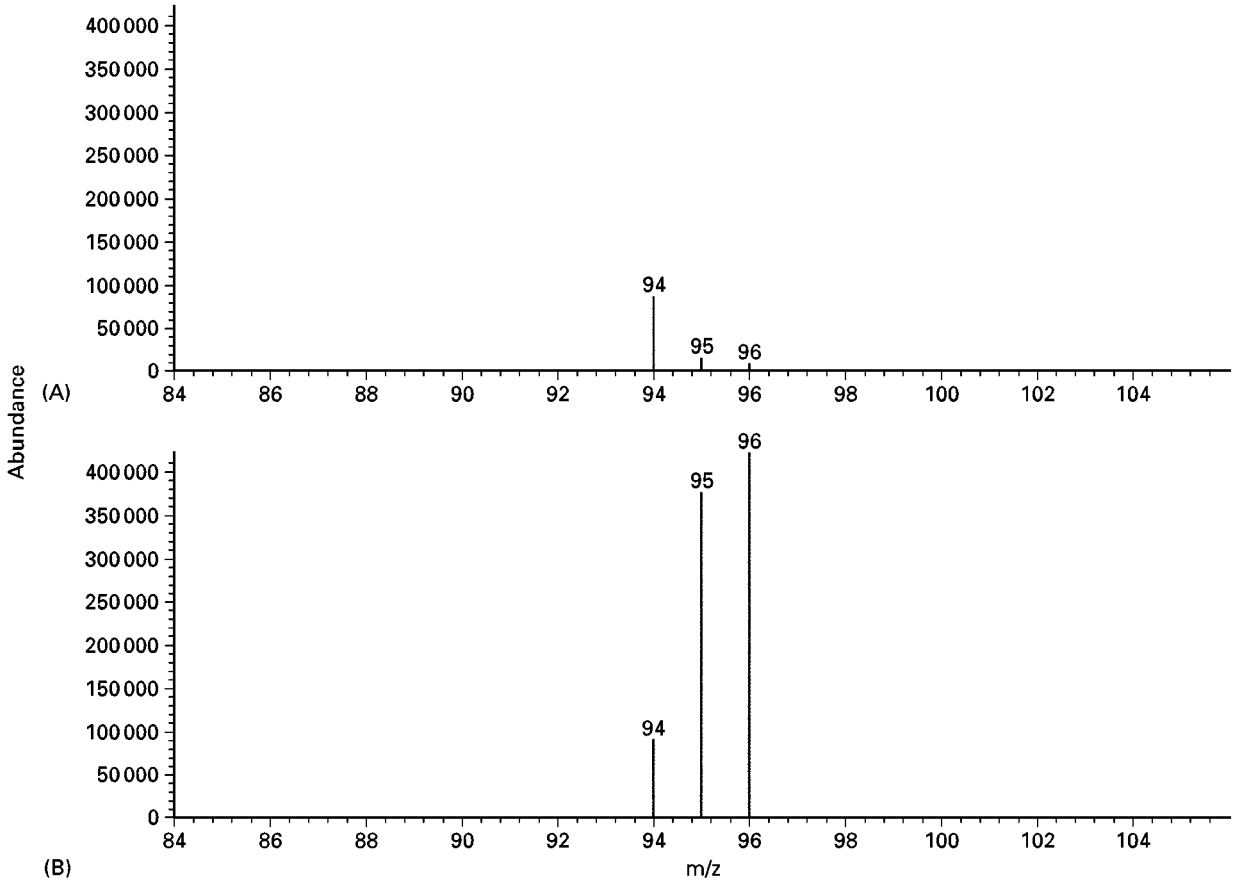

Figure 9 Selected ion-monitoring mass spectra of methylpyrazine produced in heat-treated tobacco suspensions (A) without additive; (B) with added $^{15}NH_4OAc$.

See Colour Plate 122.

See also: **II/Chromatography: Gas:** Column Technology; Detectors: Mass Spectrometry; Headspace Gas Chromatography; Historical Development; Theory of Gas Chromatography. **Extraction:** Solid-Phase Extraction; Solid-Phase Microextraction.

Further Reading

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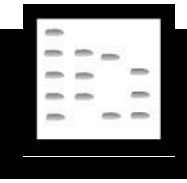
TOXICOLOGICAL ANALYSIS: LIQUID CHROMATOGRAPHY



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Introduction

With the introduction of high performance liquid chromatography (HPLC) coupled to detection systems providing spectral information (e.g. photodiode array detection and mass spectrometric detection, HPLC-DAD and LC-MS) the development of HPLC methods for broad-spectrum drug screening has attracted great interest in forensic laboratories. The information obtained by these methods is two-fold, i.e. retention data and spectral information, creating a powerful identification system. The growing literature describing HPLC as a broad-spectrum technique demonstrates its unique and essential position in toxicological investigations.

A number of important parameters in toxicological screening by HPLC include column packing material, column dimensions, detection, standardization and peak purity assessment. These topics will be treated while the applicability will be demonstrated by presentation of selected examples of general screening and specific detection of a limited number of compounds.

Column Packing Materials

Underivatized Silica

Unmodified silica can retain drugs by a weak cation exchange mechanism and was used for broad-spectrum drug screening as early as 1975. The main problem, however, with the use of underivatized silica is the substantial variability of this material. Different brands of silica and even different batches