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Short communication

Gas chromatography using a resistively heated column with mass spectrometric detection for rapid analysis of pyridine released from *Bacillus* spores☆

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Abstract

Gas chromatography using a resistively heated analytical column with full scan electron impact mass spectrometry (EI-MS) was used to detect pyridine generated from heating *Bacillus* spores in a custom designed furnace inlet, along with gasoline range aromatic (GRA) hydrocarbons representing an environmental contaminant that could interfere with detection of the biologically-derived compound. Gas phase materials from the furnace inlet were collected onto a section of cooled open tubular column, and carrier gas flow was then routed through the trapping column onto the analytical column. Both sections of column were contained within low thermal mass tubular metal sheaths, with each independently and resistively heated allowing rapid temperature ramps and cooling. An analysis time of 2 min resolved spore-derived pyridine from the other organics, and allowed identification by mass spectrum match. Throughput of 20 analyses per hour was shown to be possible with a 1-min column cool-down time between analyses.

Keywords: *Bacillus* spores; Resistive heating; Pyridine

1. Introduction

Electron impact mass spectrometry (EI-MS) is potentially useful for the detection and identification of a broad range of unknown chemicals as well as selected material derived from biological organisms [1]. For full scan 70 eV EI-MS this combined task is difficult without a separation step prior to detection; the diagnostic power inherent to full scan EI-MS is fully available with clean mass spectra from individual compounds, usually obtained through separation prior to detection. An orthogonal method such as gas chromatography/mass spectrometry (GC/MS) may be used to separate individual components in a complex mixture regardless of their origin, with mass spectra obtained from the individual peaks. Resulting EI-MS spectra for GC/MS analytes that are adequately separated can provide important information regarding the identity of initially unknown compounds present. Some problems related to the addition of gas chromatography to EI-MS detection for use outside of the laboratory include added complexity of the overall analysis scheme relative to either MS/MS or full scan MS methods without separation, and relatively lengthy run times for GC/MS analyses (typically >10 min).

The focus of this paper will be to examine an alternative to conventional gas chromatography methods allowing rapid collection of full scan EI-MS data to detect markers from a biological material and identify other environmental chemicals also present. To accomplish this, gas chromatography with a resistively heated metal column housing was used in place of a traditional air bath column oven. Rapid analyses were completed for a complex mixture of organic compounds and a chemical marker produced from bacterial spores with sample introduction in a custom-built furnace inlet. Spores from diverse microorganism species are known to contain dipicolinic acid (2,6-pyridinedicarboxylic

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acid) at relatively high proportions. The decarboxylation of this compound at high temperature predictably yields pyridine, an analyte that is amenable to GC analysis, and that has been detected previously with high temperature heating of bacterial spores in air [2,3].

Resistively heated column designs have been used previously. Lee et al. [4] proposed resistive heating of a low thermal mass GC column, and the concept was first demonstrated by Hail and Yost [5] using short aluminum-clad GC columns with mass spectrometric detection. Jain and Phillips [6] used electrically conductive paint to coat short sections of open tubular capillary GC column, and applied voltage to the coating for heating. Ehrmann et al. [7] incorporated separate temperature sensing and heating elements placed along the length of the GC column with resistive heating. van Lieshout et al. [8] used a "flash GC" system (where a resistively heated thin-walled metal sheath surrounds a typical GC column) to separate components of a petrochemical sample in about 5 min. Work by van Deursen et al. [9] used flash GC methods to separate C_{10}–C_{40} *n*-alkanes in under 1.5 min, completed analysis of mineral oil in less than 2 min, and separated four glycol ethers in under 1 min. Dallüge et al. [10] used a similar flash GC system to separate C_7–C_{30} *n*-alkanes in 0.5 min, mixtures of organophosphorus pesticides in 1.5 min, triazine compounds in 2.2 min, and 16 polycyclic aromatic compounds in 3 min. With EI-MS detection, this resistive column heating design has previously been used to separate pesticides with 3 min analysis times [11]. In addition to rapid temperature ramps, the low thermal mass of the thin tubular metal sheath allows rapid cool-down, speeding analysis of follow-on samples.

Using flash GC resistive column heating hardware from the same manufacturer as that of the system used in our work, Dallüge et al. [10] showed retention time values and GC peak area (splitless injection) reproducibility, with relative standard deviations (R.S.D.) $\leq$13 and 6%, respectively, comparable to values obtained for the same analytes using traditional GC oven heating methods. van Deursen et al. obtained retention time R.S.D. values <0.2% when heating rates did not exceed 20 °C/s [9]. Both of these groups used 5 m columns in the cited work.

2. Experimental

Non-pathogenic *Bacillus atrophaeus* (formerly *Bacillus subtilis* var. *niger*.) were obtained from the Life Sciences Division, West Desert Test Center, Dugway Proving Grounds, Dugway, UT. Prepared in 1996, the spores were present in a dry free flowing powder with a concentration of $\sim 5 \times 10^{11}$ colony forming units/g.

The resistively heated flash GC system used (Thermedics Detection, Chelmsford, MA) was integrated with a 5973 Mass Spectrometer (Agilent Technologies, Wilmington, DE) for the separation and detection of sample materials. The 5973 Mass Spectrometer is capable of scan rates fast enough to observe the narrow chromatographic peaks produced during rapid gas chromatography. A scan rate of 19.11 Hz was achieved using the mass range 40–250 *m*/*z* and suitable quadrupole duty cycle settings.

Spores were collected on the surface of a 1 mm diameter nickel probe by dipping the tip (about 2 cm) into the spore material. Following this, the probe was inserted into a custom-built furnace for spore heating and sample introduction. A 1.0 μl aliquot of complex gasoline range aromatic (GRA) mixture was injected from a 10 μl syringe directly into the furnace concurrently with insertion of the nickel probe bearing an invisible coating of spore material. These sample introduction steps lasted several seconds, and analysis of the sample proceeded immediately thereafter.

A diagram of the furnace used to thermally degrade the bacterial spores and introduce the sample to the GC/MS system is shown in Fig. 1A. Wire (22 gauge bare nickel–chromium, Consolidated Wire and Cable, Franklin Park, IL) was wound around a 0.125 in. i.d. × 1.5 in. length quartz tube. A ceramic tube (0.375 in. i.d., McMaster-Carr, Chicago, IL) was cemented over the smaller diameter tube with high temperature ceramic sealant, and the assembly was wrapped in insulation. The nickel–chromium wire was interfaced to a Variac controller connected to standard 120 V 60 Hz current. A separate thermocouple was used to measure furnace temperature. The Variac was adjusted until the temperature in the midrange of the furnace reached and held temperature at 650 ± 10 °C. Sample introduction was completed with the furnace inlet operated continuously at this temperature.

The flow and valve schematic of the GC/MS system is shown in Fig. 1B (valve set for flow of furnace inlet effluent through cold spots and sampling pump), and Fig. 1C (valve set for carrier gas flow through cold spots to the analytical column). The major components are identified in the legend for Fig. 1. The cold spots contained 10 cm lengths of 0.32 mm i.d., 0.5 μm film thickness Rtx-5 MS capillary column (Restek Corp., Bellefonte, PA), and the analytical column housed within its resistively heated metal sheath was a 12 m section of Rtx-5 capillary column with 0.25 μm film thickness and 0.25 mm i.d. (Restek Corp.). Deactivated fused silica transfer lines (0.25 mm i.d.) ran from the furnace inlet to the six-port valve kept in the statically heated valve oven (180 °C, constant temperature), from the valve to the sampling pump attachment point, and from the analytical column into the mass spectrometer (Fig. 1B and C). The mass spectrometer transfer line was kept at 200 °C. Helium was used as the carrier gas with 2 psi head pressure resulting in 45 cm/s carrier gas linear velocity (measured at 25 °C).

The cold spot focusing station is mounted on a sidewall of the valve oven and consists of a Peltier cooled aluminum block with two separate 1 mm i.d. thin-walled metal sheaths (100 mm lengths, shorter versions of the analytical column sheath described in the preceding paragraph) containing the previously described 10 cm sections of capillary GC column connected to the rotary valve. Cryogenic trapping fol-

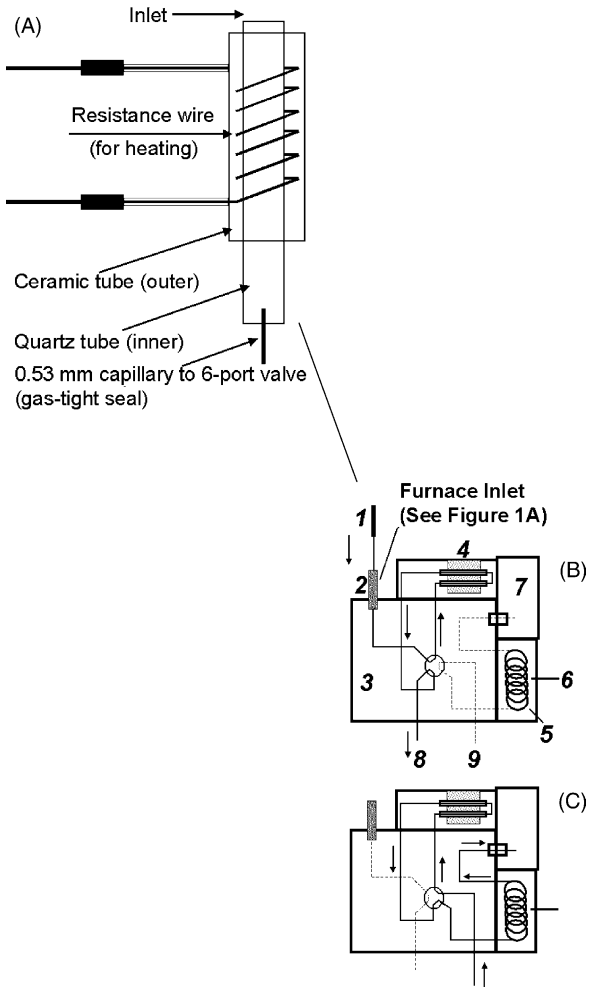

Fig. 1. (A) Furnace inlet design; (B) equipment schematic with arrows showing gas flow paths, six-port valve positioned for air flow into furnace inlet and its effluent flow through cold spots and into the sampling pump (1) representation of spore-laden probe inserted into (2) furnace inlet, (3) valve oven statically heated to 180 °C, (4) cold spots cooled to 0 °C, (5) resistively heated analytical column, (6) liquid CO_2 line for cryogenic cooling of analytical column, (7) quadrupole mass spectrometric detector (MSD), (8) membrane sampling pump (25 ml/min flow), (9) He carrier gas supply; arrows and solid line show flow path; (C) equipment schematic showing gas flow paths, six-port valve positioned for carrier gas flow through cold spots, through the analytical column, and into the mass spectrometer (solid line).

lowed by rapid resistive heating has been used by Habram and Welsch for post-column focusing of hydrocarbons sampled from air, resulting in improved chromatographic performance compared to samples collected without trapping [12].

Sampling was initiated by switching the six-port rotary valve to align sample flow through the furnace inlet to the focusing station (initially set to 0 °C) and through the membrane type sampling pump as shown in Fig. 1B. Sample material was placed into the furnace inlet, and gas phase furnace effluent compounds that could be trapped at 0 °C were collected at the cold spot. Simultaneously to the sampling step, liquid CO_2 was evaporated into the analytical column compartment, cooling the column to 0 °C. After 5 s, the rotary valve was switched again to connect the cold spots with carrier gas flow purging the analytical column and the transfer line passing through to the mass spectrometer (Fig. 1C solid line flow path) with helium for 15 s. The plug of air remaining in the cold spot lines from sampling was swept through the analytical column and the mass spectrometer while the column was maintained between 0 and 5 °C. Next, the sample material was held at the focusing station for several seconds. Current was applied to the mass spectrometer filament, and data collection commenced while the cold spots were ballistically fired to 225 °C for 20 s, focusing trapped analytes onto the analytical column held at 0 °C. The CO_2 flow was stopped and the analytical column programming proceeded to 300 °C at 164 °C/min, effecting the separation with sample components eluting into the mass spectrometer for detection and identification. Electron impact ionization (70 eV) was used.

3. Results and discussion

Pyridine and several smaller nitrogen-containing peaks were present in spore only samples (Fig. 2A) and were absent in samples analyzed with injection of GRA only (no spores). Fig. 2B shows a chromatogram produced from heating spores with concurrent injection of GRA compounds. Acceptable separation of the spore-derived pyridine and components present in or derived from the GRA mixture was completed in about 1.5 min; average peak widths of 1 s were observed, resulting in about 20 scans across a peak. The presence of aromatic aldehyde compounds (benzaldehyde, methylbenzaldehydes, and dimethylbenzaldehydes) in Fig. 2 is interesting. The same aromatic aldehyde GC/MS peaks were observed in analyses completed where GRA material was injected into the heated furnace inlet along with a clean probe (no spores). Direct injection into a split/splitless injector and analysis by GC/MS did not show these aromatic aldehyde compounds to be present in the GRA material. As the furnace inlet system heats spores and the GRA mixture in the presence of air, it is likely that these aromatic aldehydes are the result of oxidation of toluene, and various isomers of xylene, dimethyl and trimethylbenzene in the furnace inlet. Snyder et al. [2] and Thornton et al. [3] have previously shown that decarboxylation of dipicolinic acid predictably occurs in air, resulting in production of both pyridine and picolinic acid (*o*-pyridinecarboxylic acid).

The flash GC resistive heating separation technology used here could be suitable for rapid sample throughput in a laboratory setting where detection and identification of both biological and chemical components is needed, although the exact methods used to produce markers for microorganisms selected as targets would likely be different from the rather crude furnace inlet system used here. The furnace inlet did allow demonstration of the basic concepts of spore detection through identification of a chemical marker, along with con-

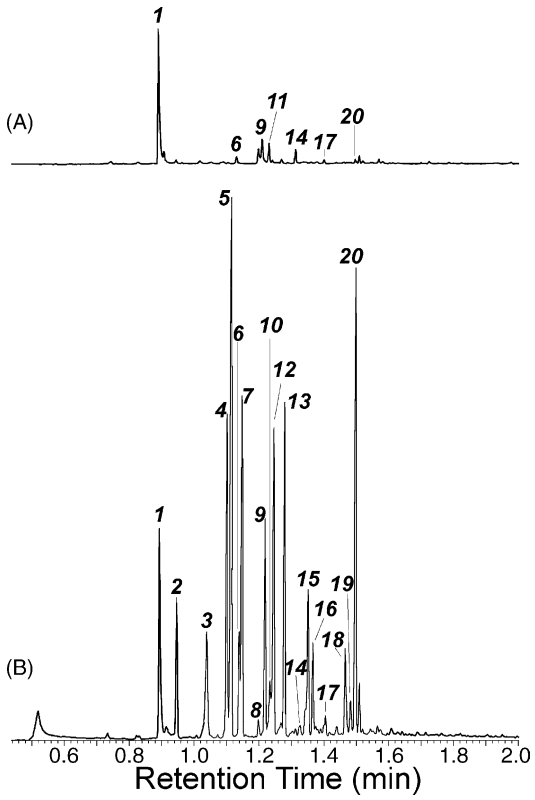

Fig. 2. (A) GC/MS total ion current chromatogram produced by analysis of spores introduced into the furnace inlet without simultaneous injection of GRA mix; (B) GC/MS total ion current chromatogram produced by analysis of spores introduced into the furnace inlet with GRA mix material injected simultaneously into the furnace inlet. All peaks were identified by mass spectrum match with reference library, except for pyridine which was identified by analysis of authentic standard material and mass spectrum/retention time match. Peak identification: (1) pyridine, (2) toluene, (3) siloxane artifact, (4) dimethylbenzene or ethylbenzene isomer, (5) dimethylbenzene or ethylbenzene isomer, (6) styrene, (7) dimethylbenzene or ethylbenzene isomer, (8) 2-pyridinecarboxaldehyde, (9) benzaldehyde, (10) phenol, (11) benzonitrile (co-elutes with peak 12), (12) trimethylbenzene isomer, (13) trimethylbenzene isomer, (14) unknown substituted pyridine, (15) methylbenzaldehyde isomer, (16) methylbenzaldehyde isomer, (17) benzylnitrile, (18) dimethylbenzaldehyde isomer, (19) dimethylbenzaldehyde isomer, (20) naphthalene.

current separation, detection, and identification of the GRA components that were selected to represent a fairly common class of environmental contaminants that could be encountered in real-world samples. Also, the gas chromatographic instrument to be used in a field setting would need to be miniaturized and engineered to consume less power.

The sampling/introduction method used here did not allow measurement of the mass of spores introduced. A logical next step in developing a separation-detection technique shown to be suitable for detection of spores along with interfering environmental contaminants should focus on detection of known airborne concentrations for both the spores and other chemicals.

Sloan et al. [13] have applied a different approach in developing a resistively heated gas chromatography column with low power consumption. They describe a resistively heated toroid assembly with low thermal mass that consists of a 15 m length of open tubular, bonded liquid phase type column wrapped in Aluminum foil. A resistive heating element (1 m of Ni wire), a temperature sensor wire (2 m of Pt wire), and ceramic insulation sleeve covering the heating element, are in contact with the GC column inside the foil wrapping. These authors completed rapid separations with quadrupole mass spectrometric detection for several mixtures using this type of column assembly, including a hydrocarbon mixture, and a mixture containing drugs of forensic interest. In addition to the low power consumption of the GC column assembly described above, its control hardware is small, lightweight, and commercially available (RVM Scientific, Santa Barbara, CA), making this a potentially useful approach to miniaturizing the type of system used in our work here for field use.

The general technique used in the present study (rapid separation of analytes with full scan EI-MS detection) could, if engineered for placement in a fieldable package, provide the basis for an important advance in simultaneous field detection and identification capabilities for both chemical and biological agents. The rapid sample throughput (about 20 samples per hour when 1 min cool-down time is included) would provide on-site information in a timely fashion for chemicals with suitable physical properties. Quick access to chemical and biological exposure information is very important in a number of fields where human exposures are of concern, including military, civil defense, and health care settings. As a drawback to this approach, relatively frequent replacement of analytical columns and other components in the system (as currently configured) may be required. This will likely be the result of high sample throughput resulting from rapid column heating and cooling times, as well as from the likely presence of water trapped in the cold spots following production of pyridine from spores in the furnace inlet.

4. Conclusion

The general concept of separation prior to detection and identification of biologically-derived material by EI-MS analysis was demonstrated using laboratory-based instrumentation. The major components of a complex mixture of gasoline range aromatics representing a possible interfering environmental mixture were successfully separated from one another as well as from pyridine produced by decarboxylation of dipicolinic acid present in *Bacillus* spores. The production of the spore marker compound occurred in a furnace inlet maintained at 650 °C with air present, and the complex organic mixture was also introduced into the same inlet. The analytical separation occurred on a 12-m length open tubular gas chromatography column housed within a resistively heated low thermal mass metal sheath. Detection was by full scan electron impact mass spec-

trometric detection. An analysis time of 2 min was more than adequate to elute up to naphthalene from the gasoline range aromatics, and throughput of 20 analyses per hour would be possible allowing for a 1-min column cool-down time between analyses. Field deployment of the techniques demonstrated would require substantial engineering and further refinement in order to miniaturize the system, and eliminate components in the currently designed and built system not amenable to field use.

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