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# Automated static headspace sampler for gas chromatography

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**Abstract**

A new automated static headspace thermal desorption system is described. This system allows for total control of the autosampler–gas chromatographic system, analog to digital conversion and data storage, prepurge functions to eliminate cross sample contamination and multiple modes of sample delivery. The system's ease of gas flow valve control and timing allows for optimal manipulation of carrier and headspace improving resolution. Larger-scale data acquisition and digital-to-analog control of the autosampler functions are discussed. System performance is illustrated using biological and environmental samples. The system's qualitative and quantitative performance was examined using statistical measures of retention time data and standard error for analyte responses.

## 1. Introduction

Static headspace (SHS) or equilibrium headspace gas chromatography (GC) methods are based upon the distribution of volatile and semivolatile sample constituents into the volume surrounding the containerized sample [1,2]. This method provides a simple means of sample preparation that separates the volatile and semivolatile constituents from the solid or liquid sample matrix. Typically the sample is weighed or measured into a sealed container with a septum and then heated to liberate volatile and semivolatile sample constituents. If the container volume and temperature are held constant an equilibrium develops between the vapor and condensed state of the analytes [3,4]. Quantitative measures are possible when the temperature of the vial is greater than the ambient temperature, the pressure of the vial is equal to or greater than the column inlet pressure, and the pressure of the vial is equal to or greater than the ambient pressure [2,4]. Under these conditions the headspace is sampled and the area produced by the analyte(s) in the headspace subsample is proportional to the concentration in the headspace [2].

The distribution of the analyte between the sample and the headspace vapor phase of the vial is described by the distribution coefficient, $K$. $K$ represents the ratio of analyte in the sample (S) divided by the analyte which is in the gas (G) or vapor phase.

$$K = c_S / c_G$$

where $c_S$ and $c_G$ are the equilibrium concentrations of the analyte in the sample and the headspace of the vial, respectively. $K$ is constant under the equilibrium conditions and proportional to the peak area and the concentration of the analyte in the sample [2].

The qualitative use of this technique has a

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general acceptance for the facile separation and identification of volatile and semivolatile hydrocarbons in liquid and solid matrices. The use of headspace GC methods by such agencies and uniform test method bodies as the US Environmental Protection Agency, American Society for Testing and Materials, International Standard Organization and Association of Official Analytical Chemists is evidence of the growing popularity of this technique. Although SHS–GC analysis is gaining acceptance as a qualitative tool, there has been debate on the suitability of SHS–GC as a quantitative methodology for solid matrices [5,6].

The interest in SHS–GC for solid and liquid matrices is evident by the plethora of recent technical papers demonstrating quantitative approaches. The use of quantitative SHS methods has replaced less specific photometric methods for the analysis of residual solvents in pharmaceutical products as a rapid and sensitive test [7]. Quantitative methods have been developed using internal and external standardization methods as well as the methods of standard additions [4]. Using first-order kinetics, Kolb and Ettre [8] recently developed a theoretical basis to support empirically derived absolute quantitative analysis for the multiple headspace extraction (MHE) method. In practice MHE makes use of multiple injections of the same equilibrated sample and uses interpolation methods to derive absolute quantities of analyte(s). The technical control requirements for MHE as well as non-equilibrium methods [9] are possible with our system.

The device discussed here is characterized by a data system which integrates the control of an autosampler's mechanical functions, the capture of chromatographic data, valve-mediated injection methods including multiple headspace extractions, actuation of gas chromatograph transistor–transistor logic (TTL) start and ready states, and peripheral devices.

## 2. Methods

The static equilibrium autosampling–GC–data system is driven by an IBM-type personal computer (PC). The system components consist of a sequence controller, analog-to-digital converter (ADC), and eight solenoids that are activated by digital-to-analog converters (DACs) (SRI, Torrence, CA, USA [10]) that control three mechanical–analytical subsystems. The solenoids used for this project are two- or three-way solenoid valves which are available in either normally on or normally off configurations. The strategic configuration of the solenoids allows for the simple control of the headspace system. SRI PeakSimple [10] software mediates all electrical and mechanical functions. An overview of the complete system is given in Fig. 1. One of the analytical subsystems controls the carrier gas and the sampling of the headspace (Fig. 2). This is accomplished by two three-way solenoid valves

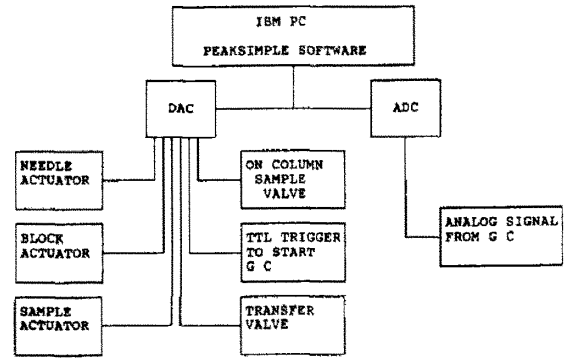


Fig. 1. Schematic overview of computer-mediated autosampler.

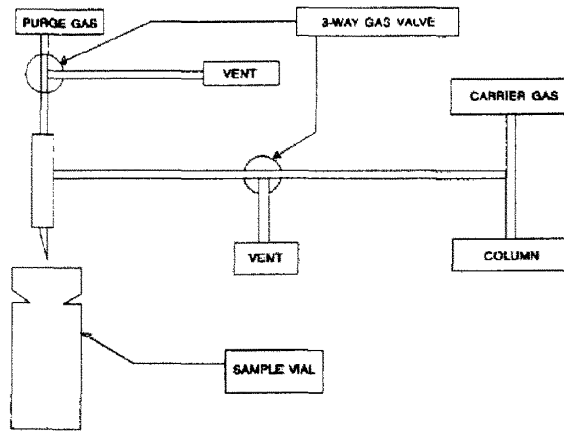


Fig. 2. Computer-mediated solenoid system for carrier and sampling gases.

and a proprietary sampling needle that are controlled by the DAC. The control of mechanical devices that manipulate the sampling needle and the sample vial positioning functions constitute a second subsystem. The manipulation of these mechanical devices is achieved with pneumatic actuators that are controlled by three-way solenoid gas valves. The actuation of the gas chromatograph TTL start function, file storage, control of run time autosampler functions, and integrator function comprise the third subsystem.

The autosampler consists of a pneumatically actuated aluminum sled that obtains the samples and places them in line with a pneumatically actuated sampling needle. The sled is thermostatically controlled and provides a dwell area for static equilibrium to take place. Sample degradation is minimized by the use of deactivated fused silica, PTFE and 313 stainless steel for all components that conduct the sample to the gas chromatograph.

The sequential control of the static equilibrium autosampler functions, data integrator, and the GC system is controlled by the data system and related interface hardware. The autosampler valve sequencing was initially configured to simulate a manual injection of a headspace sample onto the GC system with the carrier gas constantly flowing through the system. The electronic solenoid approach that is employed to load and control sample flow is a new method for sample delivery. Traditional techniques use mechanical methods to sample the headspace. We found that the use of stop carrier flow improved peak shape and chromatographic resolution. The actuation of the sample flow solenoid before actuating the on column solenoid to the gas chromatograph further improved resolution.

The sampling sequence is shown in Fig. 3. The sequence of operations of the autosampler consists of a period of sample equilibration in which the containerized sample is heated. During equilibration the sampling needle is not positioned over the sample septa (Fig. 3A). The equilibrated sample is then moved into position by the actuating pneumatic cylinder which lowers the vial into the sample holder of the heated block deletion. The sample vial is then

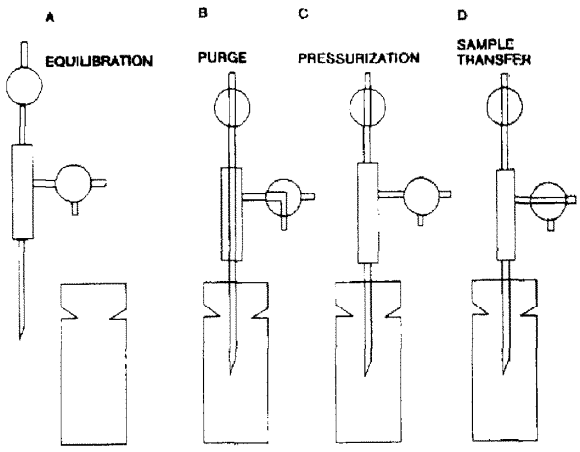


Fig. 3. Equilibration and injection sequence. For A–D, see text.

positioned for sampling (Fig. 3B). At this stage, activation of the sampling needle actuator inserts the transfer needle into the headspace of the sample vial. The autosampler system is now capable of a number of functions such as sample prepressurization (Fig. 3C), transfer line purge (Fig. 3B) or injection of the sample (Fig. 3D).

In this study, a portion of the headspace is purged through the transfer line to eliminate possible residues from prior samples. This is accomplished by the activation of the vent port of the injection valve and the activation of the sample transfer gas solenoid as depicted in Fig. 3B eliminating intersample contamination. The subsequent flow and valve states are tabulated in Table 1, showing the timed events comprising the various states the autosampler undergoes; these events are: standby flow path, purge, sampling and data acquisition. An optimized sequence timed events program is also included in Table 1.

The physical interface between the autosampler and the gas chromatograph consists of a three-way solenoid at the terminus of a deactivated fused-silica transfer line that is coupled directly to the analytical column. The autosampler operational conditions are given in Table 2.

The gas chromatograph is initiated by the computer interface card that has been modified to provide a TTL trigger to start the chromato-

Table 1
Automated run parameters

| Timed event | Time (min) |
|---|---|
| Trap solenoid activated | 0.01 |
| Sample sled solenoid activated | 0.03 |
| Sample sled solenoid deactivated | 0.05 |
| Transfer needle solenoid activated | 0.06 |
| Gas transfer solenoid purge activated | 0.08 |
| Gas transfer solenoid purge deactivated | 0.10 |
| Zero zero integrator baseline | 0.80 |
| Gas transfer solenoidactivated | 1.22 |
| Column flow solenoid activated | 1.24 |
| Column flow solenoid deactivated | 1.32 |
| Gas transfer solenoid deactivated | 1.34 |
| GC temperature cycle initiated | 1.48 |
| Transfer needle solenoid deactivated | 2.00 |
| Current sample is ejected | 2.20 |
| Data acquisition terminated | 15.00 |
| GC reequilibrates for next run | 15.00–27.00 |
| Terminate current run and cycle to next run | 27.00 |

graph once the sample has been transferred to the analytical column. The integrator subsystem consists of pre/postdata acquisition as well as run time data acquisition; integration parameters are controlled through the data system. The data acquired from each run was stored in an ASCII format as a digital voltage output file and a report output file containing sample identification, program parameters, retention time, area, peak height and concentration data. The report output file was transferred to a Lotus 123 version 2.1 [11] spreadsheet program that extracts retention and area data and subsequently unifies the data for import into the statistical program [12].

The system reported here differs from the conventional systems reported by Ettre and Kolb [2] in that: (1) the sample headspace may equilibrate along the entire conduit to the gas chromatograph, (2) multiple sequential injections are possible during the same run or separate runs, (3) either augmented or diverted carrier flows are possible, (4) integrated computer control of chromatographic, integrator and autosampler functions, (5) finer control of both sampling periods, volumes, sample introduction, and (6) a non-mechanical computer-mediated sampling method is used which allows for more flexible sampling methods.

Table 2
Autosampler conditions

| Parameter | Value |
|---|---|
| Transfer line temperature | 150°C |
| Transfer line solenoid | 180°C |
| Sled and sample temperature | 100°C |
| Sample equilibrium time | 27 min |
| Sample transfer flow-rate | 30 ml/min |

The system was tested on terpenes and coumarin compounds stored in specialized leaf hairs called glandular trichomes of *Artemisia tridentata* ssp. *tridentata* and *Artemisia tridentata* ssp. *vaseyana* (basin and mountain sagebrush). The system was also evaluated using various petroleum products in soil matrices.

Analysis of terpenes using liquid extractions [13] and headspace analysis have been previously reported [14]. Analysis of coumarin type compounds using SHS derivatization is a new approach that utilizes the temperature and equilibration time to chemically derivatize nonvolatile coumarins into their volatile methylated analogues. Separation of the terpenes and coumarin derivatives was accomplished using two capillary columns with differing phase polarity. The first column was an immobilized polyethylene glycol (30 m × 530 $\mu$m, film thickness $d_f = 3$ $\mu$m). The second column contained immobilized dimethysilicone (30 m × 530 $\mu$m, $d_f = 5$ $\mu$m) and was connected to a low-dead-volume quartz union. Relative quantitative results were obtained for known and unknown terpene components by computing the ratio of mass and area of the internal standard (ethylbenzene) peak area to the unknown terpene component. This quantitation is based upon the assumption that hydrocarbons of the same mass will provide detector responses that are approximately the same when using the flame ionization detector [14]. Terpene standards were run in the same manner to determine retention times for peak identification. The GC conditions were optimized for high sample throughput (ca. 6

Table 3
GC conditions

| Parameter | Value |
|---|---|
| Injector temperature | 200°C |
| Detector temperature | 300°C |
| Initial oven temperature | 80°C |
| Final oven temperature | 180°C |
| Oven ramp | 8°C/min |
| Carrier gas flow (hydrogen) | 44 cm/s |
| Make up gas flow (nitrogen) | 30 ml/min |
| Flame ionization detector (hydrogen) | 40 ml/min |
| Flame ionization detector (air) | 300 ml/min |
| Electrometer range | 1 |
| Electrometer attenuation | 64 |

samples/h). The GC conditions are tabulated in Table 3.

## 3. Results

One advantage of the automated headspace–GC–data system is the reproducibility and precision of the integrated system. The 95% confidence intervals for the internal standard retention times variance is less than the sampling time window of the integrator, i.e. 1 s. In order to evaluate the reproducibility of the system, 200 ng of isopropylbenzene standards ($n = 10$) were analyzed and the peak area measures used to determine the mean, standard deviation, variance and standard error (Table 4). These data indicate that the system reproducibility, accuracy and precision is on par with other ancillary automated GC devices and as such is suitable for quantitative studies.

One of the test materials consisted of leaves from a single plant that had three distinct leaf morphologies based upon lobes on the leaf.

Table 4
Isopropyl benzene, 200 ng, $n = 10$

| Test | Result |
|---|---|
| Mean | 198.8 ng |
| Standard error | 0.0186 |

Leaves that are not distinctly lobed are termed entire; leaves divided into two lobes or three lobes are termed bilobed and trilobed, respectively. An example of these features is seen in the chromatograms of various leaf morphs (i.e. entire, trilobed) that produce unique characteristic terpene chromatograms. An example of the selectivity of the system is shown in the analysis of two distinct leaf forms from the same plant as illustrated in Fig. 4. The upper chromatogram for the entire leaf came from the flowering stalk (inflorescence) and has a distinct chromatogram compared to the vegetative leaf from the same plant and branch. Fig. 5 illustrates the utility of the method to explore the time or seasonal expression of terpene and related volatile and semivolatile compounds. The system provides for rapid distinction of the two subspecies and the resulting hybrid (Fig. 6). The typical sample preparation time which consists of sample mass determination and addition of the internal standards can be manually executed in less than 2 min. With respect to sensitivity, the static headspace method reported here detected in excess of 110 components compared to 14 compounds reported in earlier studies [12,15–17].

We have demonstrated the feasibility of on-line SHS derivatization. In this approach many of the unwanted or partially derivatized reaction products are eliminated by sampling the non-volatile products that are successfully converted to volatile analogues. In this demonstration 1 $\mu$l of a 80% bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) cocktail is added to the vial with the leaf. Coumarin-type compounds present on the leaf surface react with the vaporized cocktail. Fig. 7 illustrates the use of this technique. Fig. 7a is a reference chromatogram of the leaf prior to the addition of the derivatization agent. Fig. 7c illustrates headspace derivatization of coumarin, 9-hydroxycoumarin and 2,7-dihydroxycoumarin standards. Fig. 7b illustrates the derivatization of leaf surface coumarins. It is interesting to note that the terpenes are derivatized to a volatile product.

The system was used for the analysis of petroleum-derived hydrocarbons. In this analysis di-

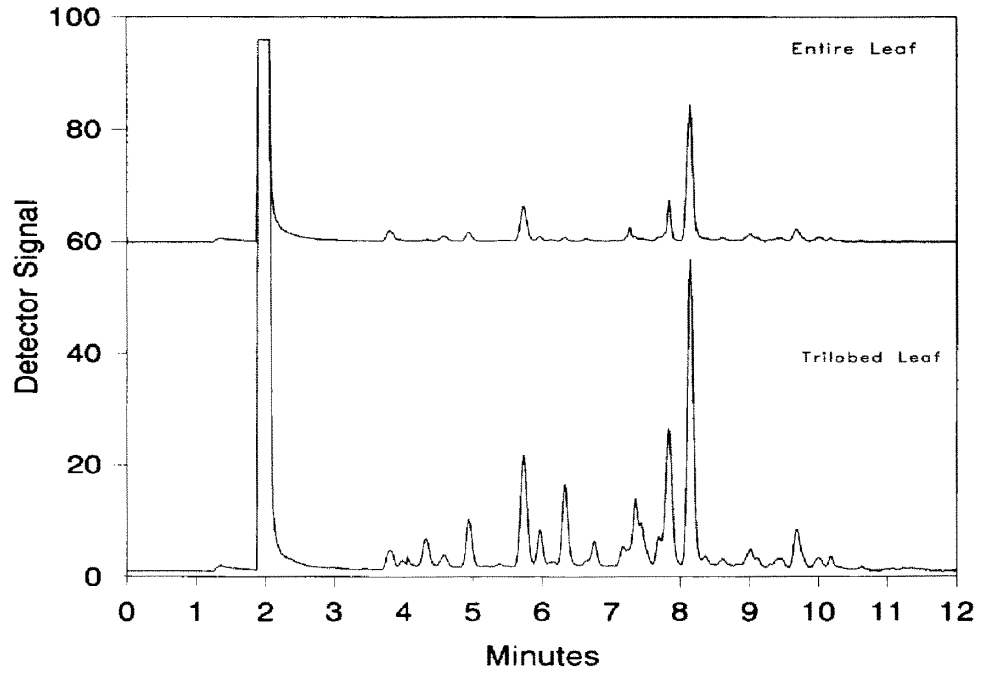


Fig. 4. Terpene chromatograms from trilobed and entire leaf morphs. Basin plant 79, branch 1.

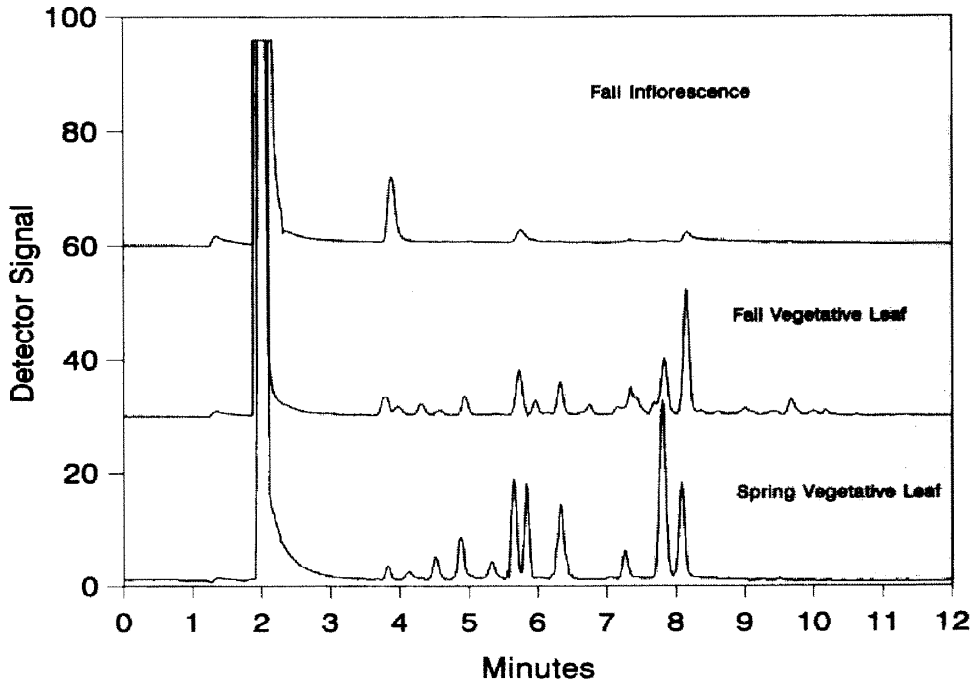


Fig. 5. Seasonal synthesis of terpenes. Hybrid plant 225, branch 1.

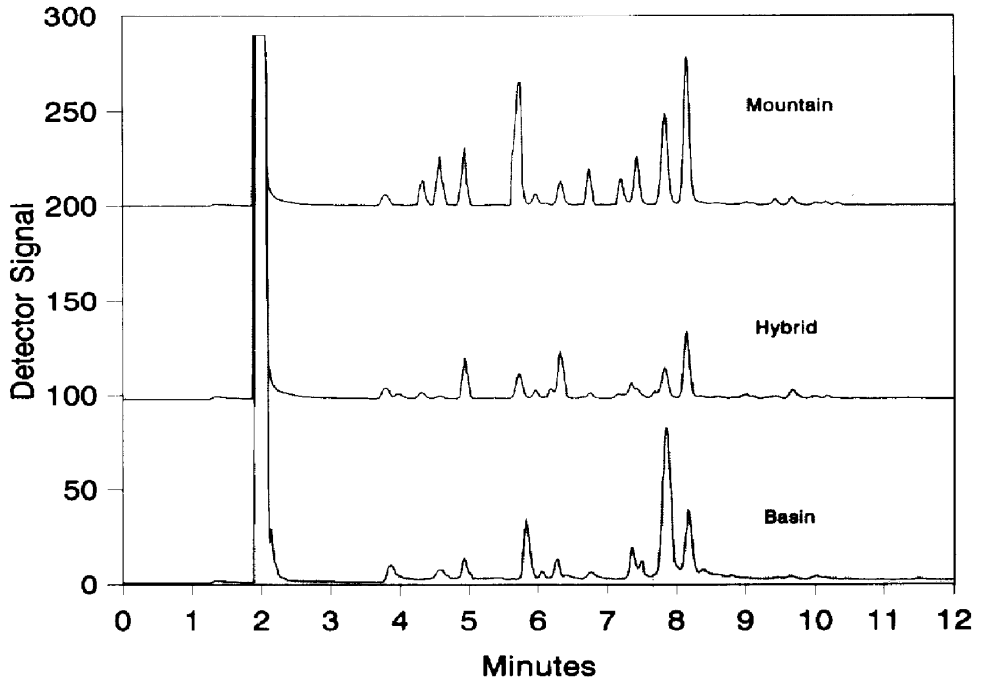


Fig. 6. Composite chromatograms illustrating the distinct terpene expressions of parental subspecies and the hybrid.

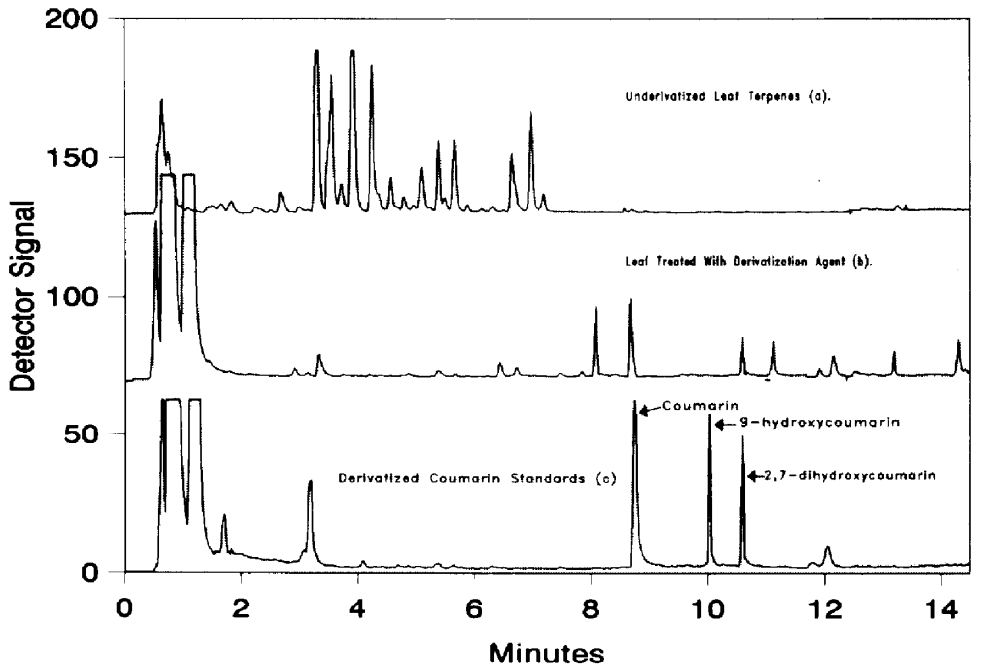


Fig. 7. Example of on-line derivatization of coumarin and related compounds.

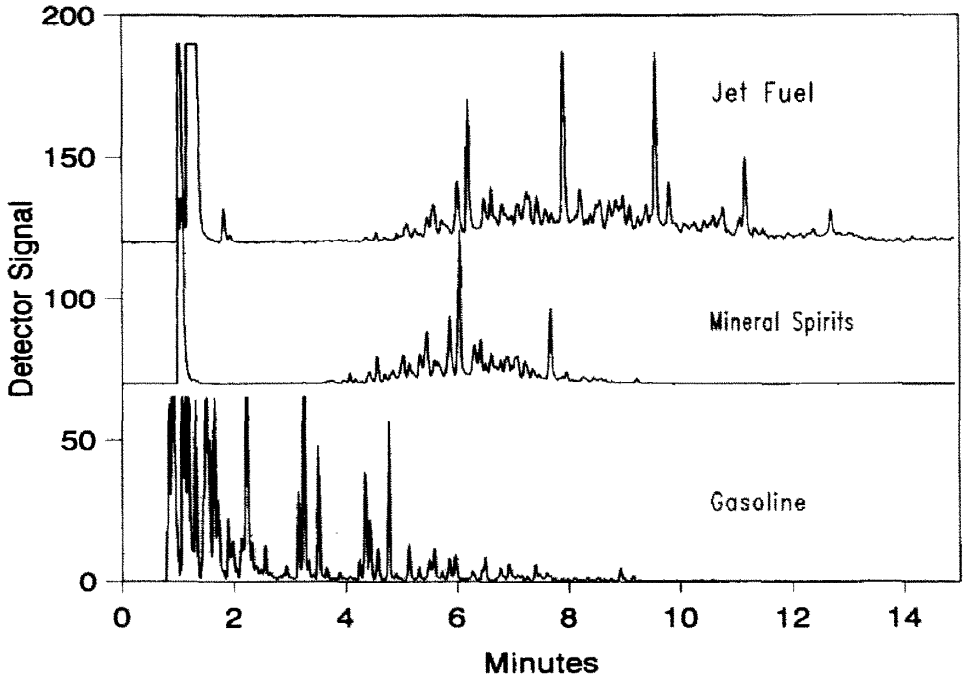


Fig. 8. Composite chromatograms of petroleum products.

lute fuel standards were either analyzed directly or in the presence of an environmental matrix (i.e. soil or debris). Fig. 8 illustrates a composite chromatogram of common fuels and their distinct chromatographic patterns.

## 4. Discussion and conclusion

The method presented provides an automated analytical tool that is a reproducible, selective, sensitive and labor-saving approach for the quantitation of volatile, semivolatile and nonvolatile hydrocarbons. The system has the advantage of high sensitivity and resolution coupled with simplicity in design and implementation. The use of an integrated headspace autosampling system allows for the precise control of several chromatographic and mechanical functions to be carried out in a routine and reproducible manner. The data indicates that precise qualitative and quantitative information can be obtained by this technique.

The system represents an extension of previous headspace sampling systems with the additional functions of flexible sampling protocols, MHE, carrier gas flow interruption and sample purge provisions.

We have shown the utility and functions of an integrated automated headspace–GC–data system that can differentiate terpenes of varying leaf morphs in close proximity. This implies a difference in biosynthetic pathways based upon leaf morphology. Similarly, we show differentiation of terpene chromatograms with respect to season, using leaves from the same plant and branch. We also demonstrate the feasibility of on-line derivatization of coumarin-type compounds using this system. An example of petrochemical differentiation (Fig. 8) illustrates the utility of this method for fuel identification and analyses.

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