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COMPUTER-CONTROLLED MASS FRAGMENTOGRAPHY WITH DIGITAL SIGNAL PROCESSING

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SUMMARY

A system for multiple ion detection has been developed in which both the control of the gas chromatograph–mass spectrometer (GC–MS) and signal processing are confined to the computer. The computer focuses on a certain mass by adding to the accelerating voltage of the GC–MS a voltage from a digital to analog converter and booster amplifier.

The digital treatment of the signals offers more satisfactory control of the baseline and gain and at the same time allows expansion of the number of channels without hardware modification.

The sampled data are continually displayed on the oscilloscope of the computer and all questions and answers are observed on the oscilloscope. The operator can set a baseline level, sample rate and gain for each channel.

The data and calculated results are displayed on the oscilloscope and can be presented as hard copy on the teletype or plotter.

The usefulness of the new system is demonstrated with regard to the metabolism of pentachlorophenol.

INTRODUCTION

By using a mass spectrometer (MS) as a selective gas chromatographic (GC) detector, multiple ion species can be detected simultaneously with great sensitivity. This technique was first used by Sweeley *et al.*[1]. Further technical developments have been described both for magnetic instruments[2–7] and for quadrupoles[8–10]. All of these systems have used analog filters, different mass channels and sequence control units to select the accelerating voltages.

The development reported here concerns the total computerization of the process, where the GC–MS is controlled by the computer and the signal processing is confined to the computer and carried out digitally. An example of the usefulness of the technique is described with regard to the metabolism of pentachlorophenol in the rat.

MASS FRAGMENTOGRAPHY EQUIPMENT

An LKB 9000 combined gas chromatograph–mass spectrometer (GS–MS) was used. This instrument was interfaced to a PDP-12 computer with 8K of core memory, a real-time clock (Digital Equipment Corp.) and a digital plotter (Houston Omnigraph).

SYSTEM DESCRIPTION

Hardware

The interface hardware system consists of a voltage control (I), a baseline control (II) and a gain control (III), which are used sequentially for all channels (Fig. 1).

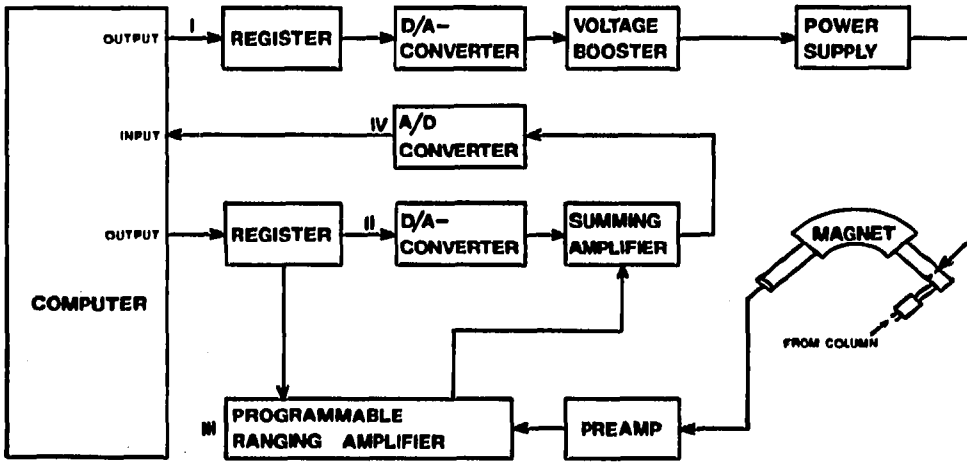

Fig. 1. Schematic diagram of computer-controlled mass fragmentography with digital signal processing.

The accelerating voltage for the mass spectrometer is controlled by the computer through a 14-bit digital-to-analog (D/A) converter and a voltage booster located in the voltage control unit (Fig. 1, I). The computer focuses on a certain mass by adding to the accelerating voltage of the mass spectrometer a voltage ranging from 0 to 1000 V that allows a mass range of 30% (calculated on the lowest mass) to be covered.

The baseline unit permits computer-controlled baseline adjustments in order to balance out any signal caused by column bleed and to bring the baselines of the different channels to the same level. This is accomplished by the addition of a d.c. voltage from an 8-bit D/A converter to the analog signal (Fig. 1, II).

The analog signal from the electron multiplier amplifier of the gas chromatograph–mass spectrometer is processed by a 6-bit programmable gain-ranging amplifier (Fig. 1, III). The use of this latter amplifier permits a more accurate determination of the selected mass area and permits a large dynamic range of the analog

mass spectrometer signal. This analog signal is converted by a 10-bit analog-to-digital converter (Fig. 1, IV) into digital information, which is stored in the computer. The accelerating voltage and the analog input parameters (gain and baseline) are simultaneously loaded into the respective control units by the software.

Software

A program that samples simultaneously a user-specified number of different masses and records the resulting data is the basis of this system. The data are continually displayed on the oscilloscope of a PDP-12 computer and all questions and answers are observed on the oscilloscope.

The system consists of the software floating-point package, the user program display routines, interface control routines and data buffers. The user parameters specify the mass, baseline level, sample rate and a gain for each channel. These parameters are accepted by the user program as described in the analytical procedure and used by the other routines for the control of the interface unit and the collection of data.

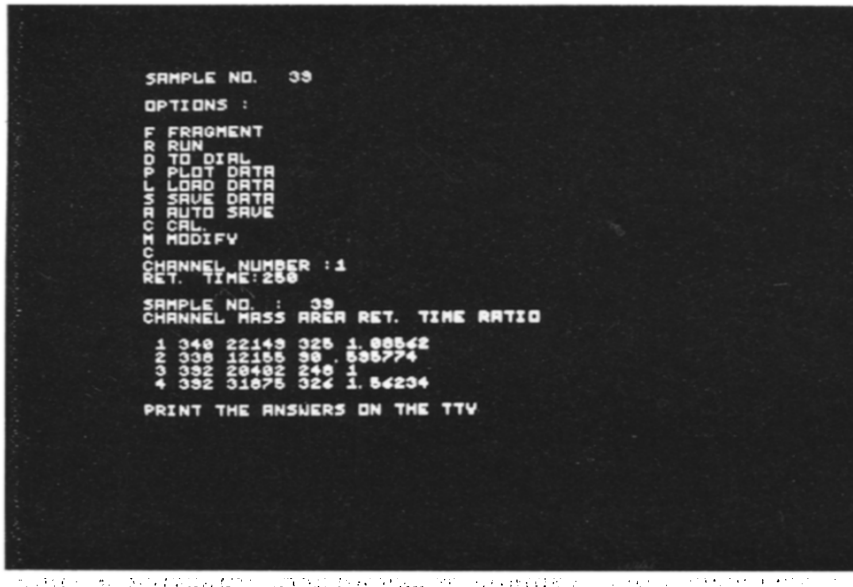

Fig. 2. Options displayed on the oscilloscope.

The operator has several options to choose from on the oscilloscope (Fig. 2):

F—FRAGMENT is used to collect data from the mass spectrometer and is followed by a request for the user parameters, as described later.

R—RUN is used to load and start a user program from the system library.

D—TO DIAL is used to return to the monitor.

P—PLOT permits a plot of the displayed data.

L—LOAD is used to retrieve previously stored data.

S —SAVE is used to store data on the magnetic tape.
A —AUTO is used to set a program mode to store the data automatically on magnetic tape.
C —CAL. is used to re-calculate peak areas and peak area ratios with new retention times.
M —MODIFY is used for background subtraction and for treatment of data.

After the specification of user parameters, the different channel curves are displayed on the oscilloscope as data are collected (Fig. 3). The data collection starts

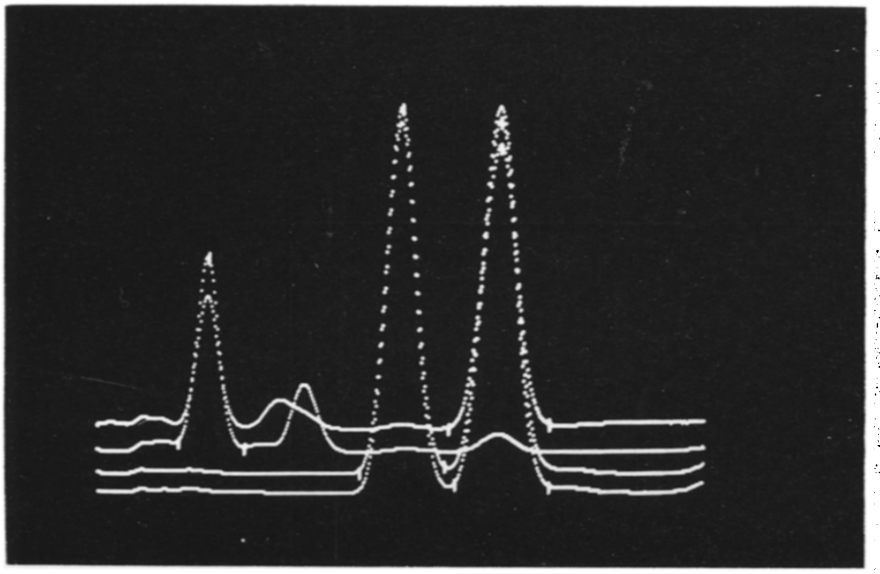

Fig. 3. Mass fragmentogram, displayed on the oscilloscope, obtained from derivatives of material extracted from rat urine. The major peaks are PCP, TCP and TCH. The mass spectrometer was set to detect *m/e* 338, 340 and 392 (two channels).

after a user-specified delay and continues until the data buffer is full or the user terminates the data collection. Determination of peak areas, peak heights and retention times occurs immediately after scanning is completed, following a spike rejection algorithm and curve smoothing[11]. The collected data are scanned according to the user-specified retention times. The peak areas or peak heights and retention times are displayed for each channel with interchannel peak area or height ratios. The data calculation can also be printed on the teletype. Background subtraction and baseline adjustments can be carried out on the collected data after collection. The system provides facilities for filing data under a sample number manually or automatically by sequential file numbers. Once the data have been stored on tape, the operator can recall any set of data for revaluation, etc. A library of user programs permits off-line plotting of groups of data automatically, or tabulations of comparisons between data sets. A sample of a user program for plotting the entire contents of the data tape is shown below.

```
100 PRINT "FIRST SAMPLE NUMBER",
110 INPUT Z
120 PRINT "LAST SAMPLE NUMBER",
130 INPUT U
140 FOR K = Z TO U STEP 1
150 REMARK READ IN THE DATA
160 TREAD K
170 REMARK PRINT THE CALCULATIONS FIRST
180 GOSUB 500
190 REMARK NOW PLOT THE DATA
200 PLOT -1
210 NEXT K
240 END
```

APPLICATION

Standard solutions

Five standard solutions were prepared by adding 100 μg/ml of tetrachloropyrocatechol (TCP) and 20–100 μg/ml each of pentachlorophenol (PCP) and tetrachlorohydroquinone (TCH) to urine collected from unexposed rats. A 1-ml volume of urine was then boiled for 2 h with 1 ml of concentrated hydrochloric acid and extracted four times with 1 ml of diethyl ether. The combined organic phase was evaporated to dryness and the residue dissolved in 0.3 ml of redistilled diethyl ether and 0.2 ml of N,O-bis(trimethylsilyl)acetamide (BSA). A 0.1–1.0-μl volume of the solution was injected into the GC–MS. Pentachlorophenol was given i.p. to 200–250-g male rats (Sprague–Dawley) in single doses of 10 mg/kg. The urine (pH not controlled) was collected at 0° in continuous nitrogen-flushed vessels. A 100-μg amount of tetrachloropyrocatechol was added to 1 ml of urine and the urine was subjected to the same extraction procedure as described above.

Analytical procedure

The components were separated on a silanized glass column packed with 5% SE-52 on Chromosorb W (60–80 mesh). The temperature of the oven and injection port were maintained at 200° and 240°, respectively. The flow-rate of the carrier gas (helium) was 20 ml/min. The ionizing potential was 50 eV and the trap current 60 μA, and the temperature of the ion source was 270°.

The mass numbers *m/e* 338, 340 and 392 were chosen for the analysis. Pentachlorophenol will respond at *m/e* 338 and 340, tetrachlorohydroquinone at *m/e* 340 and 392 and tetrachloropyrocatechol only at *m/e* 392. Two channels were focused on *m/e* 392 in order to calculate the response to tetrachlorohydroquinone at both *m/e* 340 and 392. These parameters were introduced into the program by answering the questions displayed on the oscilloscope.

The ratio of the peak heights or peak areas of pentachlorophenol (*m/e* 338) or tetrachlorohydroquinone (*m/e* 340, 392) to the internal standard tetrachloropyrocatechol (*m/e* 392) was calculated and plotted against the concentrations of pentachlorophenol and tetrachlorohydroquinone, respectively. New standard curves were

prepared each day. The amount of pentachlorophenol and tetrachlorohydroquinone in urine sample was calculated by extrapolation from the standard curves.

RESULTS

Internal standard

An internal standard is supposed to compensate for losses during extraction, differences in injection volume and evaporation during storage. Under the conditions used, tetrachloropyrocatechol was found to be suitable as its retention time did not coincide with those of other pesticides and/or impurities. A plotted recording from the analysis of a urine extract is shown in Fig. 4.

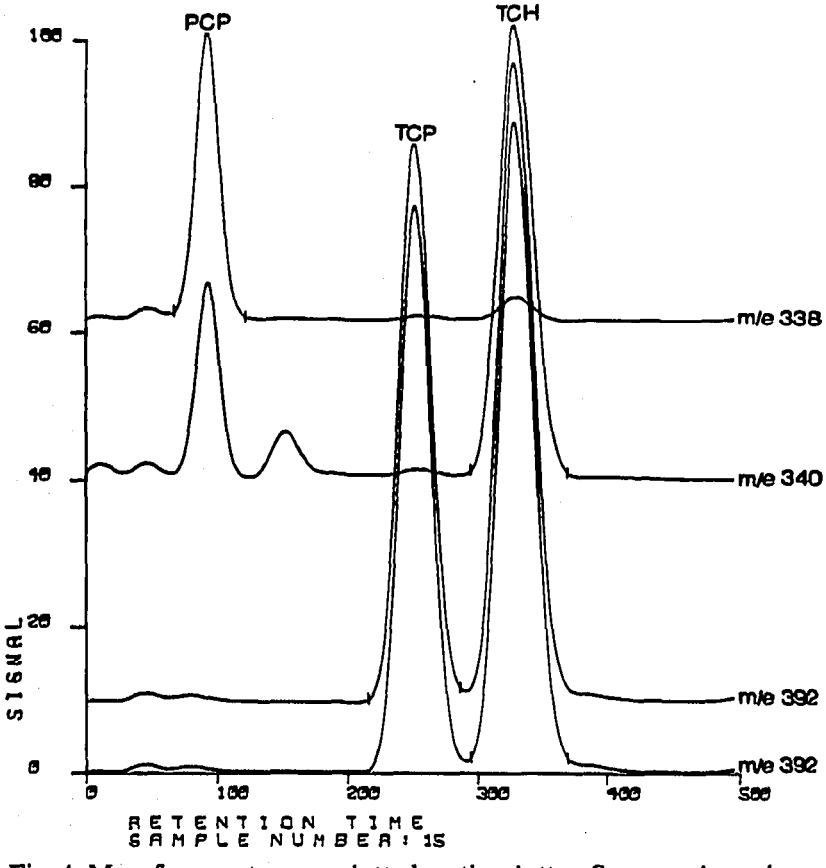

Fig. 4. Mass fragmentogram plotted on the plotter. Same peaks and mass numbers as in Fig. 3.

Standard curves

Standard curves for pentachlorophenol (*m/e* 338) and tetrachlorohydroquinone (*m/e* 340, 392) are shown in Figs. 5 and 6, obtained from calculations based on the peak height ratio and the peak area ratio, respectively. The slopes of the curves were found to be approximately the same from day to day.

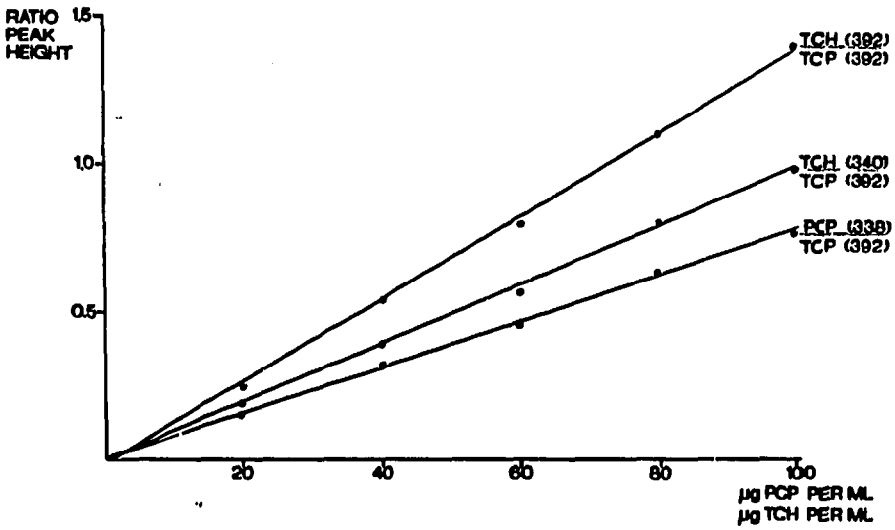

Fig. 5. Standard curve for the quantitative determination of PCP and TCH in rat urine by using the peak height ratios.

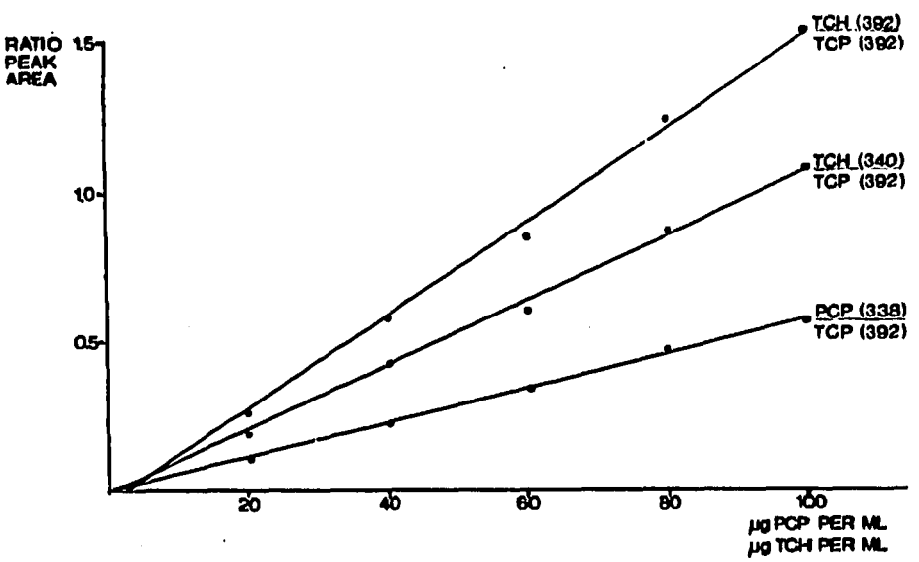

Fig. 6. Standard curve for the quantitative determination for PCP and TCH in rat urine by using the peak area ratios.

Urine excretion

The cumulative excretion of pentachlorophenol and its metabolite tetrachlorohydroquinone over a period of 72 h is shown in Fig. 7. The results are means for four rats and are in good agreement with those of total excreted radioactivity after administration of ^{14}C-labelled pentachlorophenol[12].

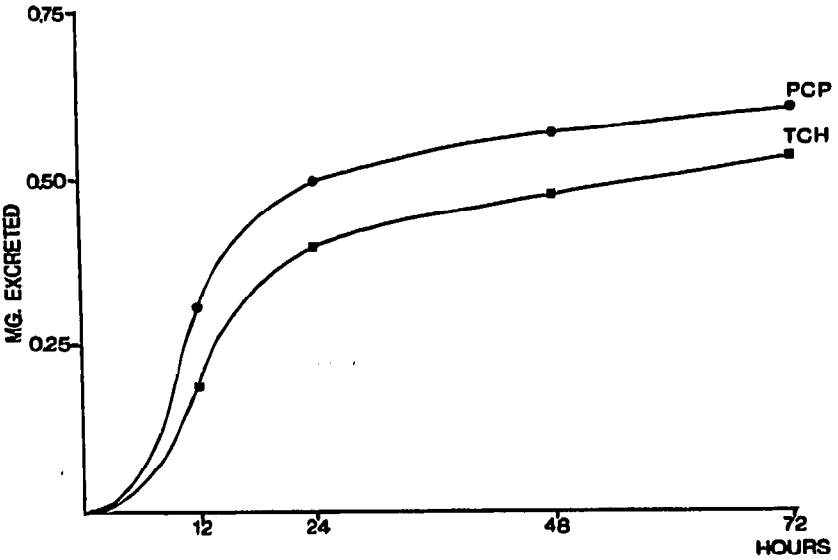

Fig. 7. Cumulative excretion of PCP and TCH in urine during 72 h after i.p. administration of PCP (10 mg/kg). Average from four rats.

DISCUSSION

Although previous techniques for multiple ion detection (MID) have been used with advantage in qualitative and quantitative mass fragmentography, the approach described in this paper offers many advantages. The MID described by Hammar and Hessling[2] has a mass range of 20% and that described by Hammar[6] a mass range of 30% of the lowest mass within which different masses can be focused. The accelerating voltages required to bring the selected masses into focus were added to the basic accelerating voltage. The curves for individual masses were made continuous by the utilization of a sample-hold circuit for each mass channel and by using one galvanometer per channel and individual channel controls for the amplification of the ion signals.

The MID system described in this paper has a mass range of 30% calculated on the lowest mass. However, in contrast to previous systems, the accelerating voltage is digitally controlled by the computer over a D/A converter. One of the main advantages is the digital signal treatment, which offers a more satisfactory control of baseline and gain and the possibility of computation of retention times and peak ratios. The system is not limited to hardware-implemented channels and therefore permits expansion of the number of channels without hardware modification. The number of channels is dependent only on mass storage limitation and the data collection rate. The system permits observations of the peaks on the oscilloscope as they emerge from the gas chromatograph.

Furthermore, the data and the calculations are presented as hard copy on the teletype. All data as well as the calculated peak values and ratios are stored on inexpensive magnetic tape for future use. The system permits calculations based on

both peak areas and peak heights; the latter seems to be more satisfactory for quantitation in biological samples.

Mass fragmentography has in recent years emerged as an important technique for the study of both drugs and biologically occurring compounds. It offers special advantages in the study of metabolism and is a specific means of detection and quantitation in the picogram to nanogram range[13]. It is hoped that the system described in this paper will facilitate considerably the use of mass fragmentography.

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