Z. Rechtsmedizin 71, 134—138 (1972)

Determination of Ethyl Alcohol by Computerized Mass Chromatography

R. Bonnichsen, B. Hedfjäll, and R. Ryhage

Government Laboratory for Forensic Chemistry, Stockholm
Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm (Sweden)

Received May 16, 1972

Summary. A combination of gas chromatograph-mass spectrometer has been used for the qualitative and quantitative analysis of ethyl alcohol in aqueous solution. An on line computer continuously acquires and stores information and controls the scan of the mass spectrometer. The results are calculated from mass numbers 31, 45, the molecular ion 46 and from the retention time. The computer is also programmed to calculate the concentration of ethyl alcohol in mixtures with other low molecular weight compounds. Alcohol samples in the concentration range of 0.02 to 0.3% can be determined with an accuracy of better than ±5%.

Zusammenfassung. Die Anwendung einer kombinierten gaschromatographisch-massenspektrographischen Methode für die qualitative und quantitative Bestimmung von Alkohol in wäßrigen Lösungen wird beschrieben. Die Auswahl der Massenspektren erfolgt durch einen Computer, der kontinuierlich mit Informationen gespeist wird. Die Ergebnisse werden aus m/e 31, m/e 45, Molekulion 46 und den Retentionszeiten kalkuliert. Eine Bestimmung der Alkoholkonzentration ist durch geeignete Programmierung auch dann möglich, wenn andere leichtflüchtige, niedermolekulare Substanzen vorhanden sind. Alkoholkonzentrationen von 0,2 bis 3% können mit einer Genauigkeit von mindestens ±5% bestimmt werden.

Key word: Ethyl alcohol, Determination by computerized Mass Chromatography.

Introduction

Modern forensic chemistry uses mostly gas chromatography, alone or in combination with an enzymatic method (ADH), for the determination of alcohol in biological samples (1—4). However, the combination of gas chromatography and mass spectrometry fulfills almost all demands for specific and accurate alcohol determinations. A recently published paper (5) describes such a combination for the qualitative and quantitative determination of ethyl alcohol. The results were obtained by the continuous study of the three mass numbers m/e 31, 45 and 46 during the time the eluate passed the ion source. The quantitation was based on the ion current of the molecule ion M = 46 and the quality was checked by the retention time and the intensity ratio of m/e 31:m/45 + 46. In this work, a computer was used for processing the mass spectral data of all masses recorded although only the three most characteristic peaks m/e 31, 45 and 46 were evaluated and used for identification and quantitative determination of ethyl alcohol. A maximum of ten other mass numbers are used for the identification of methanol, aceton and acetaldehyde. These compounds may be present in biological fluids after consumption of impure ethyl alcohol or technical alcohols.

Method

A combined gas chromatograph-mass spectrometer type LKB 9000 with a 20% polywax 4000 column 1.5 m × 2 mm has been used to separate and analyse ethyl alcohol. The signal from the electron multiplier is fed to the analog input of an IBM 1800 computer and sampled at a frequency of 8 to 12 kc. Fig. 1 shows a compressed flow chart of the processing system. At the beginning of each scan of the mass spectrometer the computer calculates the noise level and adds a fixed value to this. The result will be used to set an electronic threshold via digital output on the computer. All values above this threshold are collected and evaluated in the desired form. An electronic mass marker is used to establish the mass number. The 16-bits value in BCD-code from the mass marker is detected by the digital input of the computer for each sample on the analog input. The total ion current from the mass spectro-

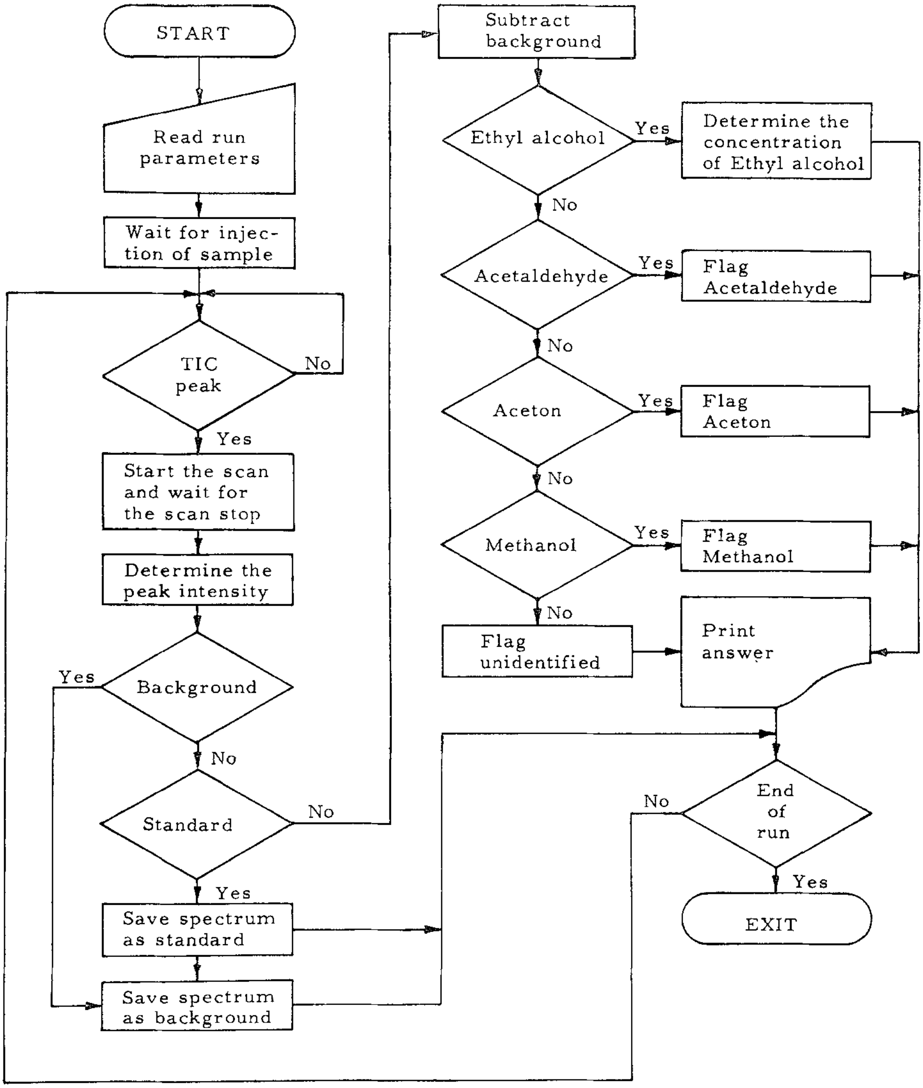

Fig. 1. Flow chart of the Ethyl program illustrating the basic steps in determination of ethyl alcohol, acetaldehyde, aceton and methanol

meter is also detected and is used to control the start of the scan of the mass spectrometer. Background spectrum must first be taken, which will be subtracted from all spectra of injected samples. The running must begin with specifying the concentration of samples for three standards, which cover the range for the unknown samples. The retention time for ethyl alcohol must also be specified. However, the specified retention time can be changed at any time during the running. The computer records the three standards and the intensities for the peaks in the spectrum will be calculated through integration of each peak. The intensities of the peaks m/e 29, 31, 32, 43, 44, 45, 46 and 58 are stored in the core memory together with the five highest peaks above m/e 28 which may be some of the mass numbers mentioned or not. Of these peaks m/e 31(-CH_2OH), m/e 45 (M-1) and m/e 46 (M) are used for identification and determination of the concentration of ethyl alcohol. Acetaldehyde is identified from peaks 29, 44, aceton from peaks 43, 58 and methanol from peaks 31, 32. The five highest peaks above 28 are used for checking if other compounds are present in the ethyl alcohol component.

In order to run the known and the unknown samples under the same condition, a part of the total ion current is detected by the computer. When the maximum intensity of each component is obtained and thereafter a few mV of decreased amplitude is detected, the computer gives out a pulse to start the scan. This decrease of the amplitude is necessary to eliminate a scan start caused of signal noise. Simultaneously with the injection of the sample an interrupt is given to the computer which starts a timer to make it to determine the retention time for respectively components. When the scan is stopped, a pulse is given to the computer to start the evaluation of the spectrum to check if criterea for ethyl alcohol is fulfilled. If the relation between the peaks does not fulfil the criterea, but the retention time is within the limits for ethyl alcohol, it is analyzed to determine whether or not the spectrum represents a mixture containing ethyl alcohol, in the event it does, the concentration of ethyl alcohol is calculated.

When either of the 2 cases is fulfilled, the sequence number and the retention time for the component together with a message, stating that this spectrum is ethyl alcohol or a mixture including ethyl alcohol with the determined concentration, is printed. When neither of these cases occur, it remains to determine whether or not the relation of the peaks corresponds to the criterea either for acetaldehyde, aceton, or methanol. If there is no correspondence, the spectrum is classified as unidentified. One of the answers is printed together with a sequence number and the retention time for the component. After the print outs the computer is ready to give a new start pulse for a new scan. The time between a scan stop and the moment the system is ready for a new scan is 1—2 sec. The injected amount of the water solution is in all cases 3 μl at an alcohol concentration between 0.01 to 0.4‰. A column temperature of 90°C gives a retention time of about 70 sec for ethyl alcohol and 50 sec further for the water peak.

Results and Discussion

To obtain an acceptable quantitative determination of ethyl alcohol by a mass spectrometric method, it is necessary to run standard samples at different concentration. The intensity measurement of these samples must be proportional to the injected sample amount of the alcohol concentration since the accuracy of the unknown sample concentration is received by interpolation between the standard samples. Instead of injecting several standards of each concentration only one of each of the three different standards are injected, but the peak intensity must be checked to be proportional to the alcohol concentration within a given value. The quantity of ethyl alcohol is determined from the intensity of the molecular ion M = 46 instead of the peaks m/e 31 (—CH_2OH) and m/e 45 (M — 1) which are of higher intensity, since the mass number m/e 46 has small background and almost no interference of peaks from other components. Ethyl alcohol is identified from the intensity relation between these peaks as well as from its retention time.

Fig. 2 shows the sum of the integrated ion current intensities of m/e 46 as a function of the ethyl alcohol concentration. The used concentrations are: 0.01,

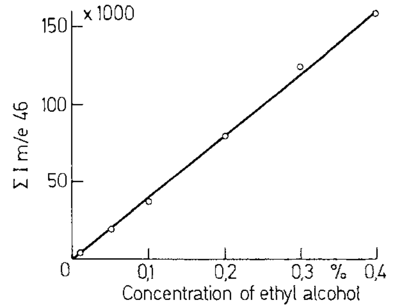

Fig. 2. Ion current intensity of m/e 46 as a function of the alcohol concentration

Table 1. *Computer print out of the results from the sample injection shown in Fig. 3*

Spectrum No. 1	at retention time =	8 sec unidentified
Spectrum No. 2	at retention time =	36 sec Acetaldehyde
Spectrum No. 3	at retention time =	47 sec Acetone
Spectrum No. 4	at retention time =	60 sec Methanol
Spectrum No. 5	at retention time =	73 sec Ethyl alcohol with concentration = 0.0196%
Spectrum No. 6	at retention time =	89 sec unidentified

0.025, 0.05, 0.10, 0.20, 0.30 and 0.40%. Of these standards three are choosen when unknown samples will be determined. Standards samples are periodically injected to check that the accuracy is within ±5% in the alcohol concentration range of 0.02 to 0.3%.

Fig. 3 shows a chromatogram of the total ion current of a mixture of four compounds in water solution and mass spectra were taken as indicated at each peak maximum. The results are given in Table 1 which shows the four identified components together with their retention times and the concentration of ethyl alcohol. It is only these four compounds which will be identified if they appear and other peaks such as 1 and 6 will not be identified and printed. The water peak is of enormous height as expected and for this reason the valve to the ion source is normally closed as soon as the peak appears which was not done in this case.

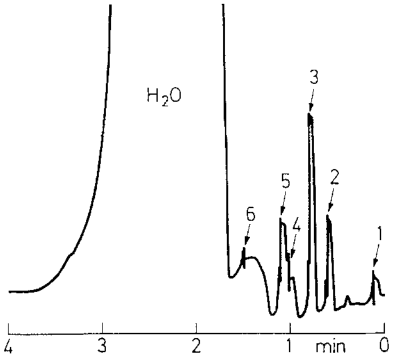

Fig. 3. Chromatogram of a mixture of 4 compounds in water solution: peak 2 acetaldehyde, 3 aceton, 4 methyl alcohol and 5 ethyl alcohol. Peaks 1 and 6 were not identified. The mixture contained 0.02% of each compound and the helium flow rate was 30 ml/min

Conclusion

The results of the computerized GC-MS system have shown that ethyl alcohol and other low molecular weight compounds could easily be analyzed using this method. Although quantitative determination has only been used on ethyl alcohol, a similar method could be used for the compounds studied and included in this paper, and for other low molecular weight compounds. By using a more sofisticated searching program even drugs and toxic compounds could be determined using a similar technic. In this work an unnecessary large mass spectrometer and computer were used for the analyzing purpose and it is clear that a much smaller system could be used for the same purpose. By using an automatic injection of the samples a complete system involving all necessary steps in the determination of ethyl alcohol could be constructed. In order to increase the capacity of the analys, two columns could be used to alternate between at the injection. In that case it should be possible to avoid the water peak to enter the mass spectrometer by switching over to a second column as soon as the alcohol peak from the first column has been recorded. The computer program size for this application is such as it will work very well in a mini-computer with 4 K words memory and without any other storage memory.

Acknowledgement. This work was supported partly by IBM in Sweden and by the Swedish Medical Research Council (project No. B71-13X-171-07C). We are indepted to Miss Ulla-Britt Gustavsson for technical assistance.

References

1. Cadman, W. J., Johns, Th.: Application of the gas chromatography in the laboratory of criminalstic. J. forens. Sci. **5**, 369 (1960).
2. Bonnichsen, R., Linturi, M.: Gas chromatographic determination of some volatile compounds in urine. Acta chem. scand. **16**, 1289 (1962).
3. Machata, G.: Über die Gaschromatographische Blutalkoholbestimmung. Blutalkohol **4**, 759 (1964).
4. Curry, A. S., Walker, G. W., Simpson, G. S.: Determination of ethanol in blood by gas chromatography. Analyst **91**, 742 (1966).
5. Bonnichsen, R., Ryhage, R.: Determination of ethyl alcohol using gas chromatography-mass spectrometry as a routine method. Blutalkohol **8**, 241 (1971).

Doc. R. Ryhage
Laboratory for Mass spectrometry
Karolinska Institutet
S-10401 Stockholm 60
Sweden

Professor Dr. R. Bonnichsen
Government Laboratory for Forensic Chemistry
S-10401 Stockholm 60
Sweden