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The determination of ethanol in aqueous solution by gas-liquid partition chromatography

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THE DETERMINATION OF ETHANOL IN AQUEOUS SOLUTION BY GAS-LIQUID PARTITION CHROMATOGRAPHY

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by

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CHAPTER I

INTRODUCTION

The present chemical and physical methods for the determination of ethyl alcohol are all subject to systematic errors derived from any of a number of interfering substances usually present in solution with the ethanol. Some of the most popular methods are slow and tedious and require a good deal of skill and technique to perform. The dichromate (2), pycnometer and refractometer methods are three such methods. The interfering substances most often present in industrial and food products are fusil oils and acetaldyhide. These substances interfere in the three methods just mentioned. It is of course possible to analyze for these substances separately and then make appropriate corrections, or to remove them before the determination is performed; but by either method the total analysis time is quite lengthy and tedious. In many cases throughout industry the determination of ethyl alcohol is a routine analysis which is usually performed by lab technicians with limited chemical background and thus lengthy and difficult methods of analysis should be minimized. The ideal method would be one which is rapid, accurate, specific and one which can be performed easily by a skilled technician.

Gas chromatography is an analytical tool which has

not yet been thoroughly investigated as a means of determining ethanol in aqueous solutions and is one which can virtually eliminate many of the undesirable features of the present chemical and physical methods. It is the purpose of this thesis to add to this investigation by presenting a moderately rapid gas chromatographic method and a high speed gas chromatographic method for this analysis and to compare these to the pycnometer and micro-dichromate methods presently used. The purpose of presenting two methods is to provide the user some choice depending on his needs and facilities.

CHAPTER II

THEORY

The basic chromatographic process is a continuous partitioning of solute between a mobile and an immobile phase. The partition approach to gas-liquid chromatography was very prominent in the early days of the technique. James and Martin applied the theoretical plate approach to the explanation of column behavior. This, of course, makes gas chromatography readily understood. Materials are separated in a gas chromatographic column by the difference in time they require in passing through it. The time each constituent spends in the gas phase must be identical. Thus separation must depend upon the time spent in the liquid phase. The partition coefficient determines how much solute is in the liquid at any time and, therefore, the over-all time spent in the liquid phase. For separation two components need slightly different partition coefficients.

This theoretical plate-partition approach was very useful and helpful. In the early days of gas chromatography it provided a very necessary feel for the significance of the column liquid and its effect on separation. Soon, however, after solving the easy problems, difficult ones were encountered. In some cases the chromatographer tried unsuccessfully to separate peaks using a variety of liquids.

He began to wonder what else he could do to achieve separation. Essentially the problem was "how to get everything of which the column was capable", in other words, column efficiency.

An equation for predicting efficiency, derived from considerations of GLC as a continuous process, is due to Van Deempter et al (5). This equation gathers the important variables contributing to efficiency together and shows the approximate effect of each. The equation yields HETP, the height equivalent to a theoretical plate, or the length of column corresponding to one equilibrium stage. It is usually expressed in centimeters.

$$\text{HETP} = 2A\ d_p + \frac{2B\ D_G}{u} + \frac{8k}{\pi^2(1+k)^2} \cdot \frac{d_f^2}{D_l}\ u$$

Where: u = rate of flow of carrier gas

$2A\ d_p$ = eddy diffusion and partical diameter

B = packing tortuosity factor

D_G = diffusion in gas phase

k = partition coefficient--ratio of solute in liquid phase to solute in gas phase

d_f = liquid film depth

D_l = diffusion in the liquid phase

The effect of temperature on efficiency is not explicitly covered by this equation, but temperature enters in through the diffusion terms and k. As temperature

increases, D_g and D_l increase, having opposite effects on HETP. The value k will decrease as more solute is forced into the gas phase, decreasing the efficiency. The net effect will depend on the magnitude of all these changes.

The data necessary to calculate HETP from the equation will generally not be available. This equation provides more aid from a qualitative point of view rather than from a quantitative view point. For example, the effect of liquid film depth may be seen by reference to the third term in the equation. The depth itself occurs as the square, but thicker films increase the amount of solute in the liquid phase as represented by k, and hence the effect is less marked than otherwise. The second term disappears at high velocity and the third term becomes higher so it can be readily seen that the flow rate of carrier gas must be optimized. It is this authors feeling that this equation is better thought of as a statement of the rules by which this game of gas chromatography is played rather than a strict quantitative mathematical formula which is useful as such.

A re-examination of the Van Deempter equation has resulted in several new proposals. All agree that a flow term inversely related to the gaseous diffusion should be included. Some workers have proposed still other terms. The area is in great need of a good deal of definitive

experimentation with careful recognition and control of all possible variables. Detailed, statistically, programmed studies seem to be necessary to permit the final refinements in the theory.

CHAPTER III

EXPERIMENTAL

Several different columns were investigated during the course of the experiment. The stationary phase was placed on the solid support in the same manner in all cases: all stationary phase materials were dissolved in chloroform. The solid support was then poured into this solution and the excess was evaporated in a rotary type evaporator. The following stationary phase and solid support combinations were investigated:

1. 10 % carbowax 4000 on 60-80 mesh teflon, 10 ft. x 3/16 in. copper
2. 3 % carbowax 20 M on 60-80 mesh glass beads, 6 ft. x 1/8 in. copper
3. 20 % carbowax 20 M on 60-80 mesh teflon, 8 ft. x 1/8 in. copper
4. 16 % carbowax 20 M on 60-80 mesh firebrick, 6 ft. x 3/16 in. copper
5. 20 % carbowax 20 M on 60-80 mesh firebrick, 8 ft. x 1/8 in. copper
6. 20 % carbowax 20 M on 60-80 firebrick, 6 ft. x 1/4 in. copper
7. 20 % carbowax 4000 on 60-80 mesh teflon, 10 ft. x 1/4 in. copper

8. 20 % carbowax 20 M on 60-80 mesh firebrick, 12 ft. x 1/8 in. copper
9. 20 % didecylphthalate on 60-80 mesh firebrick, 6 ft. x 1/4 in. copper
10. 16 % carbowax 4000 on 60-80 mesh firebrick, 9 ft. x 3/16 in. copper
11. 20 % craig polyester succinate on 60-80 mesh firebrick 6 ft. x 1/4 in.
12. 15 % carbowax 4000 on 100-120 mesh gas chrom P, 6 ft. x 1/4 in.
13. 20 % Dow-Corning high vacuum grease on 60-80 mesh firebrick, 6 ft. x 1/4 in. copper
14. 20 % carbowax 20 M on 60-80 mesh Neutraport S, 7 ft. x 3/16 in. copper
15. 20 % carbowax 20 M on 60-80 mesh firebrick, 6 ft. x 1/8 in. copper

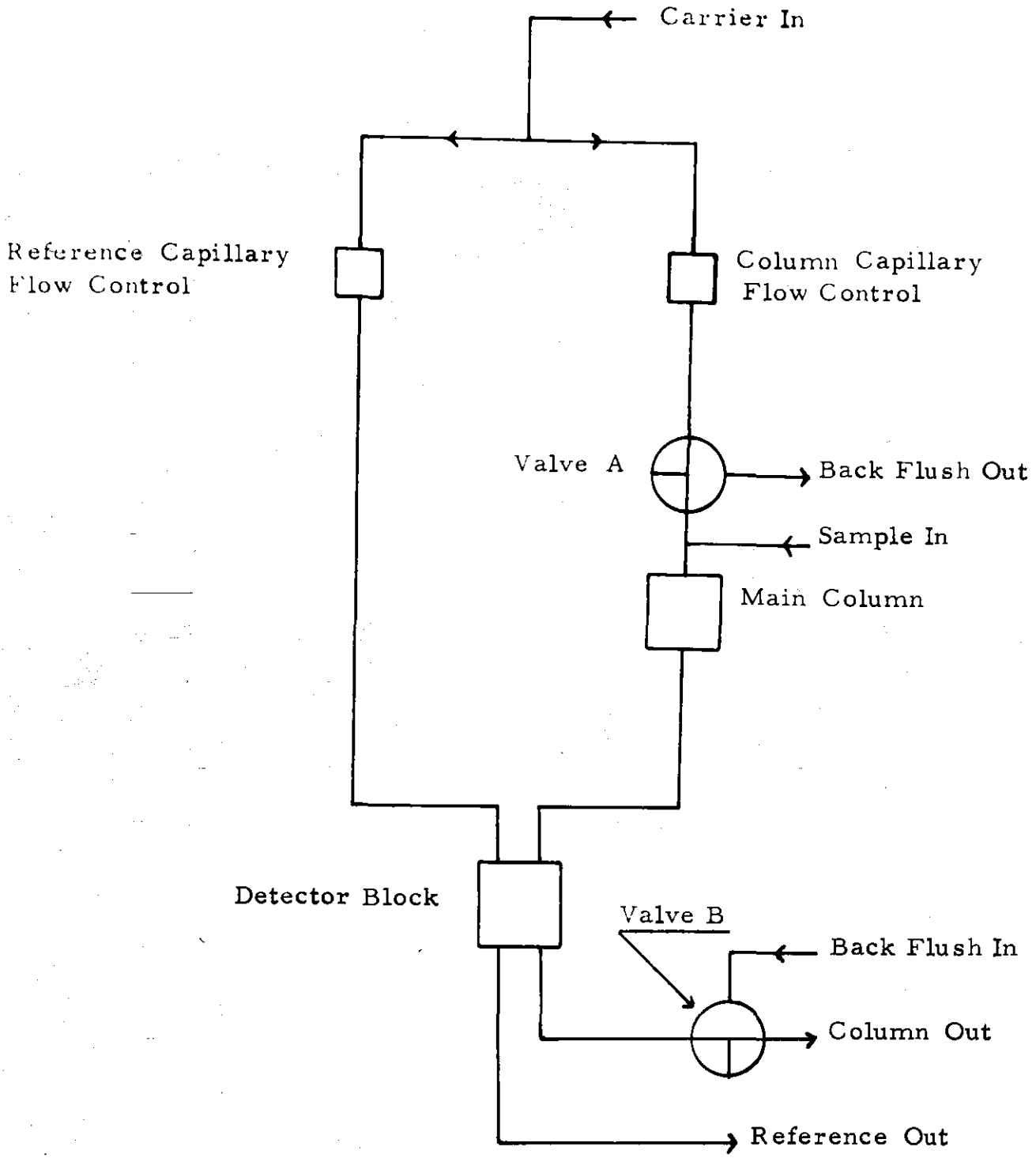
From these fifteen combinations number 3 and number 15 gave the best all around results. Many of the other columns provided good separation but the time was from 20 to 40 minutes.

The chromatograph used was a modified Beckman model GC-2. The thermal conductivity cell was replaced with a Gow-Mac thermal conductivity cell of about twenty times the sensitivity. A 12 volt battery was used as the power source to the filaments of the thermal conductivity cell, and the

temperature of the column was controlled by a variac. A high quality five millivolt recorder with a Perkin-Elmer printing electronic type integrator was used. This is a very high speed integrator, having a full scale count of 6000 counts per minute, which enables very sharp peaks to be integrated accurately. Also included was a homemade back flush system, consisting of two three-way valves in the sample side of the system.

Three different samples were used in the course of this investigation: A sample of Port wine, hereafter known as sample A; a 20 % by volume solution of ethanol in water, which will hereafter be called sample B; and a third sample, sample C, which is sample B plus 500 ppm each of acetaldyhide, n-propanol, iso-butanol, n-butanol, and active amyl alcohol. This sample was used to check all columns for complete separation of all impurities from the ethyl alcohol. Sample B was prepared from ethanol of 99.95 % purity and with volumetric apparatus which was checked by the National Bureau of Standards for calibration. This makes the 20.00 % standard actually 19.99 %.

This author prefers the "internal standard" approach to quantitative analysis by gas chromatography when there is only one component of interest being determined, and in this particular case acetone has been chosen as the internal standard.



Flow Diagram

Figure 1

The difference of the detector response to ethanol and acetone was determined in the following manner: Equal concentrations of ethanol and acetone were injected into the chromatograph and the ratios of the peak areas determined. The average ratio of ethanol to acetone after fifteen trials was 1.0703. When the sample is prepared by using 20 ml of the sample to be analyzed with 3 ml of acetone added, the acetone comprises 13.04% of the total sample injected into the chromatograph. If the ratio, 1.0703, is multiplied by the per cent acetone, 13.04; the factor, 13.96, is obtained. This is the factor used to calculate the per cent ethanol in any sample prepared in the manner just discussed.

Calculation:

$$\text{per cent ethanol by volume} = \frac{\text{area of ethanol peak}}{\text{area of acetone peak}} \times 13.96$$

Two slightly different approaches were investigated in order to provide the user some choice depending upon his particular needs and facilities. The sample is prepared in the same manner for both methods. 20 ml of the solution to be analyzed is pipetted into a 25 ml serum bottle and to this 3 ml of anhydrous reagent grade acetone is added. The serum bottle is then capped and mixed and approximately 1 microliter of this mixture is injected into the chromatograph.

For the moderate speed method the chromatograph was operating under the conditions shown in Figure 2. Under

these conditions the recorder reaches base line after the ethanol passes through the detector in approximately ten minutes. As soon as the recorder reaches base line at this point the chart drive is turned off, thus blocking any further signal to the recorder and the column is put on back flush. The column is allowed to back flush for four minutes. Hydrogen, helium or nitrogen are suitable for back flushing, although if nitrogen is used much higher pressure is needed to provide the 150 to 200 cc per minute required for efficient back flushing. After back flushing is complete, the two valves are restored to their original positions and after a few seconds are allowed for stabilization the recorder is turned on and the system is ready for another sample.

The second approach, the high speed method, is very similar to the first, the main exception being that a different column is used. This method utilizes a 6 foot by 1/8 inch column packed with carbowax 20 M on 60-80 mesh firebrick. The sample preparation and the operating variables are essentially the same, (See Figure 3). After the ethanol has passed through the detector and base line is reached, the recorder is turned off and the column is back flushed in the same manner as in the first method except that now the back flushing requires only 1.5 minutes. By performing the analysis in this manner a chromatogram is

Sample:	Sample A
Column:	8 ft. x 1/8 in. 20% Carbowax 20 M on 60-80 Mesh Teflon
Carrier Gas:	Helium
Filament Current:	270 ma.
Pressure Regulator:	30 p.s.i.
Flow Rate:	60 ml./min.
Attenuation:	x 20
Temperature:	72 °C.
Sample Size:	1 micro liter
Recorder:	5 mv.
Chart Speed:	1/2 in./min.

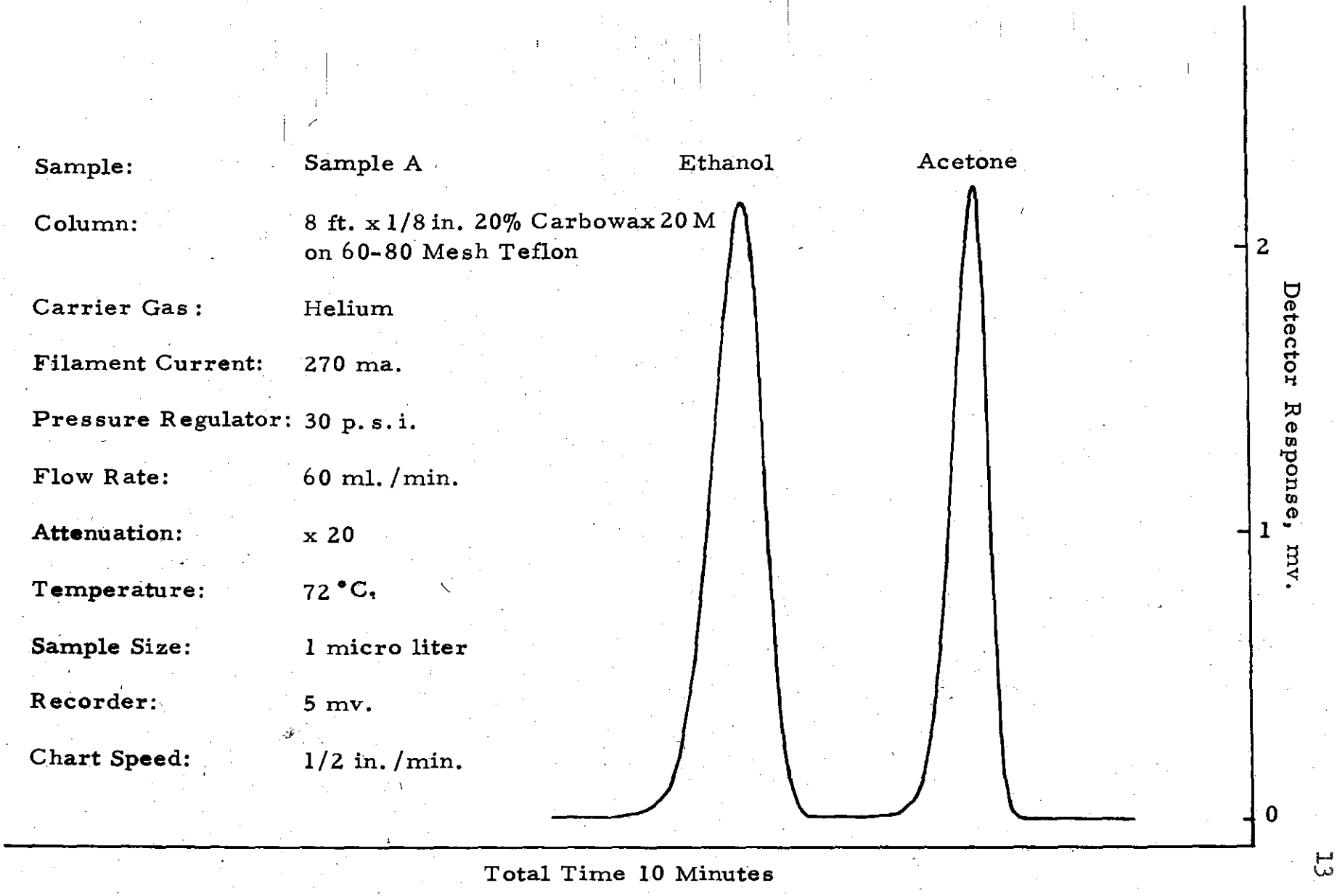


Figure 2

Sample:	Sample A
Column:	6 ft. x 1/8 in. 20% Carbowax 20M on 60-80 Mesh Firebrick
Carrier Gas:	Helium
Filament Current:	270 ma.
Pressure Regulator:	25 p. s. i.
Flow Rate:	60 ml. /min.
Attenuation:	x 20
Temperature:	72° C.
Sample Size:	1 micro liter
Recorder:	5 mv.
Chart Speed:	1/2 in. /min.

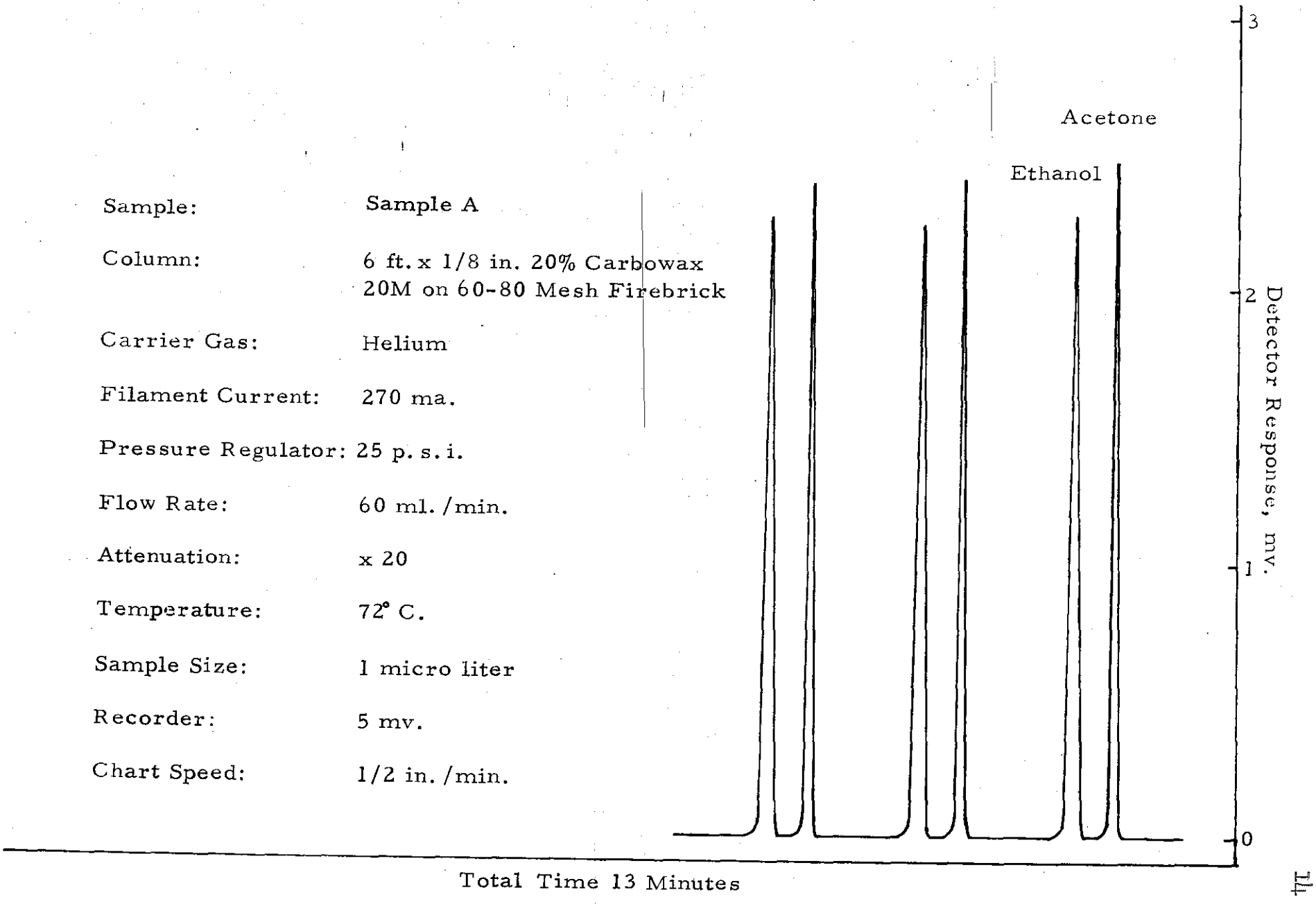


Figure 3

obtained which has the appearance of Figure 3 after three determinations are completed.

The samples analyzed by the pycnometer method were sent to an outside laboratory which has a great deal of experience in determining ethanol in wine by this method. The samples analyzed by the dichromate method were analyzed in this laboratory as it is the method presently used.

CHAPTER IV

DISCUSSION

When using the moderate speed method a chromatogram is obtained which has the appearance of Figure 2. The peaks in this figure were integrated with the electronic integrator; however, a Disc type integrator can be used. If the chart speed is also increased to 2½ inches per minute a polar planimeter can be used to integrate the areas; however, if a planimeter is used with this method, the consequent time of analysis is such that not much advantage is gained.

When using the high speed method the areas under the peaks must be integrated with a high speed integrator such as the one previously mentioned, because it takes only a few seconds for the acetone and ethanol to pass through the detector, thereby making it impractical to try to run the chart drive fast enough to get a large enough area to integrate with a planimeter. It is also possible to use a Disc type integrator to perform the integration because this type of device performs as a function of millivolt response and time and here the time is long enough providing a high speed motor is used with the Disc integrator and the chart speed is increased appropriately. When this high speed method is used it is possible to complete one analysis including the calculation of the answer every five minutes

with the accuracy shown in Table 1.

The advantages of the internal standard method over what might be called the "straight forward" method are many. First, it is not necessary to measure the sample accurately. This is a big advantage not only because it eliminates errors due to inaccurate measurement, but also because it enables a very small sample to be used, and this small sample is necessary for very rapid analysis of polar compounds (1). Secondly, it is not essential to maintain or be able to reproduce the identical temperature of the column. Relatively large temperature drift can be tolerated with no change in accuracy. The temperature needs to remain the same for only a couple of minutes during each analysis while the acetone and ethanol are passing through the detector. Thirdly, it is not necessary to be able to maintain or reproduce the same carrier gas flow rate or filament current except within relatively large limits. All that is necessary is that these variables remain constant for the same short time during each analysis. Since operating conditions do not need to be closely controlled, it is possible to use an inexpensive chromatograph. When a chromatograph is to be used for a routine analysis such as this one, any money spent on extras is much better spent on the recorder and integration device.

The converse of all the advantages to the internal

standard method are disadvantages of the "straight forward" method where the sample is injected into the chromatograph directly from the sample bottle.

The results of determining the ethanol content in samples A and B by the four methods discussed are summarized in Table 1. From the data shown it can be concluded that the dichromate method and the moderate speed gas chromatographic method compare in accuracy. They also compare in time for analysis. The pycnometer method is the least accurate and is the most time consuming. The high speed gas chromatographic method is slightly less accurate than the dichromate and moderate speed gas chromatographic methods but is more accurate than the pycnometer method and is very rapid and specific.

Table 1

Comparison of 4 Methods for the Determination of Ethanol (per cent by volume)

Sample	Trial	Pycnometer Method	Micro Dichromate Method	High Speed Gas Chromatography	Moderate Speed Gas Chromatography
A	1	19.55	19.60	19.58	19.61
A	2	19.45	19.66	19.63	19.64
A	3	19.70	19.70	19.74	19.67
A	4	19.65	19.65	19.69	19.65
A	5	19.55	19.68	19.65	19.73
	Average	19.58	19.66	19.66	19.66
	Standard Deviation	0.10	0.04	0.06	0.05
B	1	20.00	20.09	20.01	20.04
B	2	19.90	20.00	20.05	20.01
B	3	20.00	19.98	19.93	19.96
B	4	19.90	19.96	19.97	19.97
B	5	19.95	20.00	20.10	20.00
	Average	19.95	20.01	20.01	20.00
	Standard Deviation	0.07	0.05	0.06	0.04

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