CHROMATOGRAPHIC PEAK SELECTOR

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Filed: June 2, 1969

Appl. No.: 829,682

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ABSTRACT

A chromatographic apparatus and technique are described wherein a peak of interest in the output chromatogram is selected for integration by a timing circuit controlled by a preceding reference peak. The timing function starts when the sample mixture is injected into the column, and the time of appearance of the reference peak is used as the basis for computing a predicted time for appearance of the peak of interest. A similar technique is used to compute a predicted width of the peak of interest in order to control the period of integration.

33 Claims, 15 Drawing Figures
CHROMATOGRAPHIC PEAK SELECTOR

This invention relates in general to the field of chromatography. More particularly, this invention relates to improved means and techniques for determining with high precision the concentration of a component of a sample mixture injected into a chromatographic column.

Chromatography is a physical method of separation wherein a mixture of two or more components to be separated is percolated through a column containing a large surface area stationary bed of material. The stationary bed in the column is adapted to retain the components of the mixture for differing periods of time. Thus the individual components emerge at different times from the column. A detector placed at the output of the column provides an electrical signal in the form of a series of peaks each representing the concentration of a respective component. A typical detector is a well known thermal conductivity cell.

The stationary material in the column may be either in solid or liquid form while the mobile mixture may be liquid or gaseous. Hence, four fundamental forms of combination are possible for the mixture and column material. These combinations are respectively gas-solid, gas-liquid, liquid-solid and liquid-liquid.

In one chromatography technique, a continuously flowing carrier gas, such as helium, is supplied to a column packed with an inert material such as Celite which has been coated with a high boiling point organic-liquid such as dinonyl phthalate or silicone oil. A sample of the mixture to be analyzed is inserted in the carrier gas to be entrained thereby through the column. Components in the sample will have different degrees of affinity for the column materials and their retention times in the column will thus differ. Accordingly, the components in the sample will elute separately and at different times from the column.

Any variation in the composition of the gas exiting from the column caused by an eluted component from the sample will cause an unbalancing of a detector circuit at the column exit. The degree of unbalance is proportional to the concentration of the component. The detector sends a corresponding signal to a utilization device, frequently a strip chart recorder. The recorder thus draws a series of peaks which usually appear at least approximately symmetrical. The concentration of any component may be obtained by measuring the corresponding peak height, but for the most reliable and accurate results the peak area should be measured.

One serious problem in using the integration technique has been the difficulty of determining accurately when the integration should begin and end. Prior methods for accomplishing this have usually depended on a time base generator and a signal selector with adjustable controls to operate the selector at fixed points in time. Obviously, errors in the measurement will result if the integrating period used is too short for the particular peak of interest, or if the period begins too soon or ends too late so as to include a part of a preceding or succeeding peak in the integration results.

Although the length of time required for any particular component to pass through a column will be known approximately from previous calibration data, the exact time is influenced by factors such as temperature, column loading, which changes with usage, and flow rate. Prior equipment typically included control equipment to maintain the temperature and flow rate set at predetermined fixed levels, so that the analysis would always be conducted under the same conditions for which the original calibration was established. Such control equipment not only had to be quite elaborate and expensive, but it did not solve the problem of long-term variations in column loading.

In a preferred embodiment of the invention to be described hereinbelow in detail, there is provided a chromatographic instrument particularly designed to measure the concentration of a single component of an injected sample. Such an instrument is especially useful in process control applications. Another application is that of measuring the concentration of alcohol in a person's breath, in order to determine, for police purposes, whether he is intoxicated. In any such applications, the measurement must be consistently accurate. Moreover, the apparatus must be relatively simple and automatic in operation, yet it must be relatively inexpensive to manufacture.

In the described embodiment, special means are provided for accurately determining automatically the period during which the peak of interest is to be integrated. This determination is effective over a wide range of temperature, flow rate and column loading conditions prevailing during any particular analysis run. Thus, there is no need for highly elaborate control equipment for eliminating long-term drift of temperature and flow rate.

In accordance with one aspect of this feature, a reference component is supplied to the column in such fashion as to produce a reference peak prior to the peak developed by the component of interest. This reference peak has a unique characteristic permitting it to be distinguished from the other peaks automatically. Based on a measurement of the time difference between the appearance of this reference peak and the initial peak to appear in the detector output, a computation is made predicting the time of appearance of the peak of interest. Specifically, it can be shown that there will be a constant ratio between the times of appearance of the reference peak and the peak of interest, over changing conditions of flow rate and temperature. Similarly, the ratio of the width of the reference peak to the width of the peak of interest will remain substantially constant, so that by measuring the width of the reference peak, it becomes possible to automatically compute the width of the peak of interest, i.e., the length of time for the integrating action.

In the preferred embodiment, the apparatus employs a method which might be termed "plateau elution chromatography" as a means for developing a reference peak having a unique, readily recognizable characteristic. In this method, the carrier gas is purposely contaminated (doped) with a component which can dissolve in the stationary liquid phase or which can be adsorbed on the active solid support. Provided that the dopant is not present in the sample or, if present, is in lower concentration than in the carrier gas, it will be found that a reference peak will be produced at a retention time characteristic of the particular dopant and having a polarity opposite to the other peaks produced, e.g., the reference peak will be "negative" if the other peaks are considered to be "positive." Thus, the reference peak can be recognized and distinguished.
The time necessary for the reference component to pass through the column as well as the width of its peak are measured parameters used to generate a "time window" during which the detector output is identified as providing the peak of a component of interest to be analyzed. For the duration of this window the detector output is integrated to provide an accurate measure of the area of the peak of interest. The results of this integration represent the concentration of the component being analyzed.

As noted above, the time interval between an initial peak (such as air in the sample) and the reference peak will bear a constant ratio to, i.e., will be directly proportional to, the time interval between that initial peak and any selected peak of interest over changing conditions of temperature, flow or quantity of stationary phase. In some cases the initial peak occurs almost immediately after the sample is injected into the column, and as a practical matter it can be considered that the time between injection and appearance of the reference peak will be directly proportional to the time between injection and the appearance of the peak of interest. If the reference peak appears at a measured time $t_1$ with a time width of $W_1$, then the time $t_2$ for appearance of the peak of interest relates as

$$t_2/t_1 = K_1$$

and the time width $W_2$ of the peak of interest relates as

$$W_2/W_1 = K_2$$

These ratio relationships have been found to hold true over significant temperature and flow rate variations.

Based on these relationships, means are provided in the disclosed embodiment to compute automatically the starting and ending times for the period $W_2$ during which the integration of the peak of interest takes place. The integrating apparatus also includes special means to eliminate errors which might otherwise occur due to drift of the base line during integration.

Accordingly, it is an object of this invention to provide improved means and techniques for chromatographic analysis. It is a more specific object to effect an accurate integration of a signal peak produced by chromatographic methods. Other objects, aspects and advantages of the invention will be understood from the following description of a preferred embodiment of the invention considered together with the drawings wherein:

FIG. 1 is a schematic diagram of a gas chromatography instrument and system in accordance with the invention;

FIG. 2 is a waveform diagram illustrating operating waveforms of voltages at different points of the diagram of FIG. 1;

FIG. 3 is a circuit diagram of an integrator used to measure the area of the signal peak;

FIGS. 4A - 4G shows a network employed to generate timing signals for the operation of the integrator of FIG. 3;

FIGS. 5a and 5b show a timing diagram illustrating the operation of the network shown in FIG. 4;

FIG. 6 is a circuit diagram of a timer used in the embodiment of FIG. 1;

FIG. 7 is a circuit diagram of a timer used in the embodiment of FIG. 1; and

FIG. 8 is an enlarged portion of a signal waveform shown in FIG. 2.

With reference now to FIG. 1, a chromatography apparatus of the elution-partition type is shown in block diagram form. This apparatus includes a column 10 containing a suitable known material for the separation of components of a gaseous mixture. The column inlet port 12 is connected to the outlet port of a sample valve 14 which may be of known construction. The valve 14 has one input port 16 coupled to a supply of gaseous material 20 to derive a sample therefrom for analysis. A second input port 18 is coupled to a mixer 22 supplying a carrier gas, such as helium, and a dopant gas such as acetalddehyde, in a concentration of approximately 0.5 percent. The mixer 22 has input ports coupled to supplies of carrier gas 24 and dopant gas 26. Alternately the dopant can be premixed and one tank of carrier gas used.

The valve 14 is provided with an actuating lever 28 which may be manually or automatically controlled. The actuation of the lever 28 introduces a preselected quantity of the sample into the carrier gas stream for passage to the column 10. At the same time the lever actuation a pulse $t_0$ is generated from a start network 31. The sample gas inserted at the inlet port 12 of the column 10 eventually elutes from a column exit port 13 with the components separated by the column. The eluted material passes through a thermal conductivity cell 30 for the detection of the separated components and the production of electrical signals representative thereof. After passage through detector 30 the carrier gas stream and separated components are vented at an exhaust port 32.

The output of detector 30 is amplified in amplifier 34 to provide a suitable signal $g(t)$ for analysis. The output of this amplifier is applied to an integrator 36 which operates under control of a timing controller 38. The output of the integrator is applied to a utilization device 40 such as a strip chart recorder, meter or other display device.

In the chromatographic apparatus of FIG. 1, the carrier gas is doped with a preselected material, not present in the sample (or present in much smaller concentration), to cause the detector 30 to produce a reference peak having an opposite polarity from the peaks produced by other components in the sample. The reference peak in effect represents a "vacancy" in the sample, and may be unambiguously detected because of its opposite polarity characteristic. Although in the preferred embodiment the reference peak actually represents a missing component in the sample, it can be considered to have been produced by what can broadly be termed a reference component in the sample.

The waveform 42 in FIG. 2 illustrates a chromatographic signal $g(t)$ representative of a person's breath containing ethanol. Several of these peaks are of such large amplitude that they are shown in truncated form. It should be understood, however, that these peaks are in practice essentially symmetrical and continuous. A first peak 44 is produced by air in the sample, a second peak 46 by water vapor. The third peak 48 is the reference peak with an opposite polarity, and represents the absence of the preselected material used to dope the carrier gas. A fourth peak 50 represents the ethanol present in the sample. As can be seen from waveform 42, the peaks 44, 46 and 48 are large compared with the ethanol peak 50 but this is to be ex-
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pected in view of the small amount of ethanol present. The waveform 42 further has a base line 52 which as illustrated may drift downwardly or upwardly during the operation of the instrument.

The chromatograph waveform 42 commenced at a time t₄ corresponding with the time of insertion of the sample into the carrier gas stream. The air and water peaks 44–46 are detected first, followed by the reference peak 48 at time t₁. The appearance of the reference peak must be uniquely detected, in order to permit a measure of the time t₄ required for the vacancy to pass through the column, as well as its duration or "width" W₁ equal to t₂ - t₁. From this information a "window pulse" is subsequently generated commencing at time t₂ and terminating at time t₄.

Returning to FIG. 1, the start pulse t₀ is directed to a first timer unit 64 provided to measure the time required for development of the reference peak. This timer basically consists of a ramp generator the output of which, as shown by waveform 74 (FIG. 2) increases linearly with time while the reference component is in the column. In order to sense the appearance of the reference peak, the output of amplifier 34 is coupled to a reference crossing detector network 56 to which also is applied a reference signal from a reference source 58. The reference signal voltage is selected to correspond with the threshold level 54 of FIG. 2, and the network 56 produces a predetermined output signal whenever the measurement signal is below that threshold level. Thus, as the reference peak is produced by the chromatographic detector 30, the crossing detector produces a pulse of width W₁, i.e., pulse 60 in waveform 62.

The threshold level 54 is located quite low (near base line 52) on the reference peak 48 to reduce the influence of amplitude changes of the reference peak on the measured duration W₁. The reference voltage may be manually adjusted, but generally remains fixed for any particular dopant. However, it may be desirable to provide means for automatically eliminating any net base line offset signal at the start of each cycle so as to maintain a constant threshold detection level relative to the base line of the signal from the detector 30. Such offset can be introduced by a suitable bias which can automatically be made of the correct value to zeroize the output of detector 30.

The leading edge of the reference peak pulse 60 is applied to the timer 64 to cause it to reverse its direction of ramp action, i.e., to sweep downward. The slope of the downward sweep is prefixed for the component of interest. Thus when the timer output returns to zero, it will at that instant identify a second time t₂ which is proportionally related to t₁ by a predetermined proportionality factor K₁.

The output of this timer unit 64 is applied to a second threshold crossing detector 66. When the downward ramp 70 reaches zero volts, this threshold crossing detector produces a short output pulse 72. By selecting the slopes of the upward and downward going ramps, the output pulse 72 is made to occur at the desired time t₄ signaling the start of the peak of interest. The pulse 72 is used to start the integration function for the peak of interest.

In order to determine the width of W₂ of the integration window following time t₄, means are provided to measure the width W₁ of the reference peak. For this purpose, there is provided a second timer unit 86 which, like the first timer 64, makes use of a ramp generator for making time measurements. This second timer is started by the pulse t₄, and produces a linear up-sweeping signal shown in waveform 90. The subsequent pulse t₄' at the end of the reference peak stops the ramp action, and the signal level thus built up is stored for subsequent use in fixing the width of the integration window for the peak of interest. Thus, at the start of that window, time t₄, the ramp action is reversed to produce a downwardly sweeping signal form along 96. When the signal crosses zero volts, this is detected by a crossing detector 66 which thereupon produces a pulse tₛ' signifying the end of the period of integration. The slope of the downward ramp is pre-set at a fixed ratio with respect to the slope of the up ramp. Therefore, the period of integration will always be directly related to the width of the reference peak.

FIG. 6 illustrates details of the timer network 64. An operational amplifier 76 is provided with positive and negative inputs and a negative feedback capacitor 78 to provide an integration function at its output 74. A potentiometer 80 has its end terminals selectively coupled to positive and negative supply voltages through switches 82–84 to provide the respective upward and downward ramps 68–70. Switches 82 and 84 may be electronic elements such as gate insulated field effect transistors. As shown, switch 84 is closed at t₀ and opened at t₄, and switch 82 is closed at t₁ and opened at t₄. The voltage at output 74 thus swings upwardly and downwardly at a slope determined by the setting of the potentiometer 80 with the result that the downward ramp reaches zero volts at a time tₛ related to t₁ by the ratio of tₛ/t₁ = K₂ where K₂ is selected in accordance with the setting of the wiper arm 85 of the potentiometer. Switch 79 is closed at the beginning of the tₛ pulse and opened at the end of the pulse to assure that at time tₛ the voltage across the capacitor 78 is zero.

FIG. 7 illustrates a detailed circuit which will produce the waveform from network 86. Thus an operational amplifier 98 having positive and negative output terminals with positive input grounded is provided with a negative feedback capacitor 100 having a capacitance C. A switch 102 shorts capacitor 100 to provide initial voltage control (zero) over the capacitor stored voltage. The negative input terminal is coupled to the junction of a fixed resistor 104 having a resistance Rₚ, and a variable resistor 106 having a resistance Rₛ. The resistors 104 and 106 are connected respectively to negative and positive voltage sources through switches 108 and 110 respectively. The switches 108 and 110 may be electronic and are actuated under control of timing pulses as labelled in FIG. 7.

In the operation of the timer circuit 86, and with reference to the waveform 90 in FIG. 2, switch 102 is closed at time t₀ and opened at time tₛ. At time tₛ switch 108 is closed thereby permitting the output of operational amplifier to rise, charging capacitor 100 at a slope determined by (+V)/RₛC V represents the supply voltage. At time tₛ' (Wₛ later), switch 108 is opened and the output on capacitor 100 is held at level 94 since there is no discharge path. At time tₛ switch 110 is closed, thereby forcing a downward sloping ramp 96 with a slope determined by...
When the ramp 96 crosses zero volts the detector 88 issues the pulse 89 which is representative of the time \( t_1' \) when the pulse \( W_2 \) terminates.

Pulses \( t_1 \) and \( t_1' \) respectively set and reset a flip flop 112 to provide at its output 114 a wide pulse 116 representative of the desired integration window \( W_2 \).

The width \( W_2 \) of the window pulse 116 can be varied by selecting the position of the wiper arm of the variable resistor 106 in FIG. 7. This varies the resistance \( R_2 \) and correspondingly alters the constant \( K_2 \) in the relationship

\[
\frac{W_2}{W_1} = K_2
\]

In this manner the proper constants can be dialed into the instrument by varying the potentiometer 80 (FIG. 6), for \( K_1 \), and 106 (FIG. 7) for \( K_2 \).

In a typical application of the instrument of FIG. 1 for the detection and integration of the ethanol peak 50, \( K_1 \) was 0.45 and \( K_2 \) was 3.74.

Integration of the ethanol peak 50 proceeds as may be best understood with reference to FIG. 8 which shows the peaks on an enlarged scale. In general, the procedure is as follows: At time \( t_1 \) the function \( g(t) \) is sampled and the voltage \( g(t_1) \) of the base line 52 is stored. Thereafter integration proceeds on a signal which is the difference between \( g(t) \) and \( g(t_1) \). The area thus integrated equals \( -(A_1 + A_3 - A_2) \). At the end of the integration at time \( t_1' \), the base line 52 is sampled again and the difference between the base line voltages at times \( t_1 \) and \( t_1' \) is stored. Thereafter the area within the triangle formed by areas \( A_1 + A_3 + A_2 \) is determined by integrating the stored difference signal and dividing the result by two. The triangle area is effectively added to the previously integrated function to yield the net area of the peak \( A_1 + A_3 \).

FIG. 3 illustrates details of the integrator 36. The detector output signals \( g(t) \) from the amplifier 34 are coupled through a switch KA to the negative input 132 of an operational amplifier 134 having its positive input grounded. This amplifier includes a feedback capacitor 146 which, in conjunction with input resistor 146A provides linear integrating action. During the initial part of the cycle, i.e., prior to integration, capacitor 146 is effectively bypassed by a resistor 142 so that amplifier 134 serves a normal amplifying function.

The operation of the integrator 36 is dependent upon the setting of the several switches. The following description of its operation relies upon a preselected terminology for the control signals A, B, C, D, E, F, and G which respectively control the state of switches KA, KB, KC, KD, KE, and KG. A “0” indicates that the switch is in its normal state, as drawn in FIG. 3, and a “1” indicates the switch is in its alternate position. The control signals for the switches are generated as shown in FIG. 4. The switches may be conventional electronic gates. The timing relationships of the control signals A through G may be observed from FIG. 2 where a positive level represents “1,” and a zero level represents “0.”

Prior to integration, the switches are in the condition A B C D E F G 0 1 0 1 0 0 0

During this condition, the voltage is applied to input 132 of amplifier 134 which acts as a straight amplifier. Its output is inverted by a second amplifier 148 and directed through a feedback loop 138 including a third amplifier 152. The net voltage at the input to amplifier 134 is maintained at zero by negative feedback action, so that the voltage at junction 156 will be equal to \(-g(t)\). A voltage divider preferably is used in the output circuit of amplifier 152, e.g., to provide a ratio of 1,000:1, so that the voltage at the amplifier output actually will be \(-1,000 \times g(t)\). This voltage is stored in a memory capacitor 160 connected around amplifier 152. It may be noted that this feedback action prior to integration effectively zeroizes the integrator, i.e., it stores a signal value representing not only base line drift level, but also including a component responsive to any amplifier offset voltage.

At time \( t_1 \), switch KD is opened, and the signal at the output of amplifier 152 is \(-1,000 \times g(t_1)\). This value is stored in capacitor 160 since there are no current paths available to discharge capacitor 160. Thus, \( V_a = -g(t_1)\).

KC is now closed and KB is opened so that the control signals are

A B C D E F G 0 1 0 1 0 0 0

The feedback current around amplifier 134 through capacitor 146 is

\[
g(t) - g(t_1) = \frac{g(t) - g(t_2)}{R}
\]

where \( R \) is the resistance of resistors 146A and 158. This current develops a voltage across the capacitor 146 (of capacitance \( C \)) equal to

\[
\frac{1}{RC} \int_{t_2}^{t_1} (g(t) - g(t_2)) dt
\]

Since the input of amplifier 134 is maintained at ground potential (the + input is grounded), the voltage at output 135 of amplifier 134 will be equal to the voltage across capacitor 146. The waveform 186 in FIG. 2 illustrates the voltage at the output 135 of amplifier 134.

At the time \( t_1' \), the end of the integration period \( W_1 \), the switch KC is opened to stop further integration, and the charge on the capacitor 146 remains fixed at that level while certain control functions are effected to prepare for a base line correction. To this end, switches KE andKF are closed to bring in operation another feedback loop 139 including a second feedback amplifier 164 having a memory capacitor 166. The control signals are thus in the following states:

A B C D E F G 0 0 0 0 1 1

Amplifier 134 still requires, because of its grounded positive input, a zero input voltage. This requirement drives the junction 178 to a voltage \( V_a = -g(t_1') + g(t_2) \). After this voltage has been established, switch KE is opened so that the memory capacitor 166 stores the voltage \( 1,000 \times (g(t_1) - g(t_1')) \), i.e., a voltage representing the difference between the base line level at the start of integration and the base line level at the end of
integration. This difference voltage thus reflects the amount of base line drift which has occurred during integration.

The next step in preparing for the base line compensation is to zeroize feedback loop 138. For this purpose, switch KD is closed and switch KA is opened. This effectively causes junction 156 to return to zero since all other inputs to amplifier 134 are now equal to zero. Thus any amplifier offset voltages are substantially eliminated. The switch signals at this time are as follows:

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
</tbody>
</table>

Immediately after discharge of memory capacitor 160, the switch KD is opened to disconnect the second feedback path 138 from the integrating amplifier 134. The integrating capacitor 146 however retains the accumulated integral.

At this time the base line correction is commenced by applying to the input of the integrator amplifier 134 a voltage derived from memory capacitor 166 and carrying out a further integration for a period of time proportional to the period of original integration. As previously explained, the capacitor 166 stores a voltage representative of the difference between the base line levels at times $t_3$ and $t'_3$. Graphically, the correction required is to remove the area enclosed by the right-angled triangle indicated at 188 in FIG. 8 from the area originally integrated for the time period $W_3$. Hence, after the storage in capacitor 166 of the difference voltage $g(t_3) - g(t'_3)$ and the effective discharge of capacitor 160, switches KC and KG are closed to connect the stored signal of memory capacitor 166 to the input of the integrating amplifier 134. The charge on the capacitor 146 thereby is augmented (or diminished) at a rate proportional to the amount of base line drift which occurred during integration. The control signals at this time are:

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

The time period of this compensating integration is determined by the time period of the original integration “window.” However, it will be apparent that the compensation time period need not be the same as that of the original integration, providing that a corresponding change is made in the level of voltage applied to the input of the integrating amplifier during the selected time period. In one embodiment, the input voltage level was selected to be equal to 10 times the actual amount of base line voltage drift. This multiplication factor was introduced by a suitable voltage divider in the output circuit of amplifier 164, specifically by selecting the resistor 176 to be 100 times the size of resistor 180. Thus the voltage at junction 178, with switch KG closed, will be 0.1 times the voltage on memory capacitor 166; since this capacitor voltage was 1,000 times the base line drift voltage, the net output voltage will be 10 times the base line drift voltage.

With a voltage multiplication factor of 10, the time period for the compensation integration must be one-twentieth of the original period of integration, in order to produce the required amount of correction as discussed above in connection with FIG. 8. This reintegration time period is controlled, in the present embodiment, by the same timer unit 64 previously used to fix the time $t_2$, i.e., the start of the original integration window.

With further reference to FIG. 6, therefore, at time $t_2$ switch 84 was again closed to cause the output of timer 64 to ramp up as indicated by waveform 74, at line 190. The switch 84 is opened at the end of the integration ($t'_2$), and the timer thereby stores a signal level 192 corresponding to the period of integration ($W_2$).

Upon the commencement of the base line correction, at time $t_3$, a switch 118 (FIG. 6) is closed connecting the negative input of the amplifier 76 to a positive voltage source 122. This source has a voltage which is greater than that of the positive voltage source 111 by a factor of 20, determined by the desired shortening of the time for the base line correction.

The closure of switch 118 drives the output of amplifier 76 to ground along ramp 194 (FIG. 2). Upon reaching zero volts output, the threshold crossing detector 66 issues a pulse 196 at a time $t_4$. The time period $t_5 - t_4$ for base line correction is proportionally related to the integration window $W_2$. This proportional dependence upon the period $W_2$ may be appreciated from the fact that the duration of $W_2$ determines the level 192 and, consequently, the time for the subsequent downward ramp 194 to reach zero volts.

Returning to FIG. 3, the only input to amplifier 134 during the time period $t_5 - t_6$ is the difference voltage $10 (g(t_2) - g(t'_2))$ at the output of amplifier 164. The base line correction integration proceeds with this voltage for the time period $t_5 - t_6$ as indicated by waveform 186. Upon the occurrence of the $t_4$ pulse 196, the switch KG is opened to stop integration, and the amplifier output 135 at time $t_6$ represents the area of the ethanol peak 50. This final output may be recorded on a suitable strip chart instrument or the like.

FIGS. 4A through 4G and FIGS. 5A and 5B illustrate a circuit for generating the control signals A through G. These control signals are produced by using timing pulses such as $t_0$, $t_1$, $t'_1$, $t_2$, $t_3$, $t_4$, $t_5$, and $t_6$. The generation of these timing pulses has been described previously except for $t_0$, $t'_1$, $t_2$ and $t_4$. With initial reference, therefore, to FIGS. 5A and 5B the timing pulse $t'_2$ is shown connected to a network 202 from which pulses $t_0$, $t'_1$, $t_2$, and $t_4$ are generated in the order as shown in the timing diagram of FIG. 5B. These pulses may be produced by conventional delay circuits and single shot multivibrators as are well known in the art. The pulses $t_0$, $t'_1$, $t_2$, and $t_4$ occur right after the integration window when the voltage waveform 74 in FIG. 2 is held to level 192. Since this appears as a small time span, the control signals effective during this time period are shown on an enlarged time scale as is indicated at 203 in FIG. 2.

FIG. 4A includes a flip flop 204 having a set input 206 and a reset input 208. The output 210 provides the desired control signal 60 for the control of switch KA in FIG. 3. The reset signal for flip flop 204 is the pulse $t_6$ and the flip flop is set by timing pulse $t_4$ as shown by the A waveform in FIG. 2.

FIG. 4B includes a flip flop 212 which is set by pulse $t_6$ and reset by a pulse D' generated from a delay circuit 213 actuated by timing pulse $t_2$. The output of flip flop 212 is the control signal B having a waveform as shown
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in FIG. 2. The delay of the resetting of flip flop 212 avoids unwanted transients as various switches in the integrator are actuated.

FIG. 4C includes a flip flop 214 set by a pulse from an OR circuit 216 driven by timing pulses D' and t4. The flip flop 214 is reset by a pulse from OR circuit 218 coupled to timing signals t2' and t4. The output of flip flop 214 is the control signal C whose waveform appears in FIG. 2.

FIG. 4D illustrates a flip flop 220, which is set by an OR circuit 222 coupled to timing pulses t5 and t4 and is reset by the OR circuit 224 coupled to timing pulses t2 and t4. The output of the flip flop 220 is the control signal D whose waveform appears in FIG. 2.

FIG. 4E shows a flip flop 228 set by timing pulse t6 and reset by t4'. The output of the flip flop 228 is the control signal E shown in FIG. 2. In a similar manner the flip flops 230 and 232 provide at their outputs control signals F and G whose waveforms are shown in FIG. 2. The flip flop 230 is set by t4 and reset by t6. The flip flop 232 is set by t4 and reset by t6.

The utilization device 40 may be actuated at the completion of the base line correction to record the final integrated output from the amplifier 134 at time t6. Such synchronized recordation may be accomplished by coupling the t6 pulse to a strip chart recorder whose pen is momentarily enabled by pulse t4 to record the output 135 of amplifier 134.

Although a preferred embodiment of this invention has been described hereinabove in detail, it is desired to emphasize that this is for the purpose of illustrating the invention, and should not be considered as limiting thereof except as required by the prior art, it being understood that many variations within the scope of this invention can be devised by those skilled in this art to meet requirements of specific applications. For example, although the instrument described herein provides a measure of a single component of a sample mixture, it is apparent that the same techniques can be used for measuring multiple components. Other modifications will be apparent.

We claim:

1. A chromatographic apparatus comprising a column through which a sample mixture may be directed and including material adapted to separate the individual components of the mixture so that they elute from the column in predetermined sequence to be sensed by a detector which produces an output in the form of a peak-type signal responsive to the concentration of the component in the sample mixture; the improvement in said apparatus for identifying the time when a selected component of interest will elute from the column so as to permit making an accurate measurement of the signal produced thereby, comprising:

means to introduce into the column a substance to develop in said output a reference peak occurring prior to the peak of interest and having a uniquely distinguishable characteristic, the rates of passage through said column of said substance and said selected component having a predetermined relationship;

means to detect said distinguishable characteristic;

means to measure the time between a predetermined initial event and the detection of said reference peak; and

means responsive to said measured time for computing in accordance with said predetermined relationship a corresponding time when said peak of interest will be developed.

2. Apparatus as claimed in claim 1, wherein said measuring means comprises an electronic ramp generator adapted to produce a linearly varying signal until said reference peak is detected, whereby the signal level at that time represents the measured time.

3. Apparatus as claimed in claim 2, wherein said responsive means includes means for reversing said ramp generator so that it ramps back to its initial condition;

means for detecting the time when the ramp signal reaches its initial condition and for starting the integration of the peak of interest.

4. The method of chromatographic analysis comprising the steps of:

operating a chromatographic column to produce a uniquely recognizable reference peak the time of appearance of which bears a certain relationship to the time of appearance of the component of interest;

determining the time period between a predetermined initial condition and the appearance of the reference peak;

computing a second time, subsequent to and based both on said time period and said certain relationship, following which the component of interest will appear; and

producing a signal responsive to said component of interest which appears after the computed second time.

5. The method of claim 4, wherein the computation of said second time includes the step of multiplying the first time period by a predetermined constant.

6. The method of claim 4, including the steps of:

measuring the width of the reference peak;

performing a mathematical operation on said measured width to compute a predicted period of time for the width of the peak of interest; and

integrating the signal produced by the component of interest during the computed time period thereof.

7. A chromatographic apparatus comprising:

a chromatographic column means adapted for separating components in a sample mixture driven therethrough by a fluid carrier stream including a preselected reference component having a uniquely recognizable elution characteristic upon separation by the column means, the time of appearance of said reference component bearing a predetermined relationship to the time of appearance of a desired mixture component;

detecting means for sensing the emergence of components separated by the column means and for producing signals representative thereof;

means for recognizing the detected signal representative of the preselected reference component and for producing a timing signal representative of the time for the reference component to pass through the column means; and

means effectively controlled by the timing signal and said predetermined relationship for selecting the detected signal of said desired mixture component to be analyzed and for producing a signal corresponding therewith for analysis.
8. The apparatus as claimed in claim 7 and further including:
means actuated by the timing signal for integrating the mixture component detected signal for a time period related to the timing signal.
9. The apparatus as claimed in claim 8, wherein the timing signal producing means further includes:
means actuated by the detected reference component signal for generating a first pulse signal having a width related to the width of the detected reference component signal; and
means for generating a second pulse signal having a width bearing a first preselected ratio to the width of the first pulse and applying the second pulse signal to the integrating means to determine integration of the desired mixture component signal.
10. The apparatus as claimed in claim 9, wherein the second pulse signal generating means further includes:
means for initiating the second pulse signal at a time bearing a preselected ratio to the time for the reference component to pass through the column means.
11. A chromatographic apparatus comprising:
chromatographic column means for separating components in a sample mixture entrained therethrough by a carrier fluid stream;
detecting means for sensing components eluting from the column means and for producing electrical peak signals representative thereof;
means for introducing a reference fluid into the column means to identify the time when a selected component in the sample mixture will elute from the column, said reference fluid being selected to produce a uniquely recognizable reference peak signal from the detecting means at a time bearing a predetermined relationship to the time of elution of said selected component;
means based on said predetermined relationship, and actuated by the reference peak signal, for generating a control signal substantially coincident in time with the elution of the selected component from the column means; and
means actuated by the control signal for analyzing the peak of the selected component.
12. The apparatus as claimed in claim 11, wherein the reference fluid introducing means introduces the reference fluid into the carrier fluid to produce a recognizable vacancy reference peak signal from the detecting means representative of the relative vacancy in the sample mixture of the reference fluid in comparison with the carrier fluid.
13. A method of analyzing a component in a sample mixture introduced to a chromatographic column, comprising the steps of:
measuring the time required for the elution of a uniquely recognizable reference component from the chromatographic column;
computing the start time of a time period the start of which bears a predetermined relationship with the measured reference component elution time, with the computed start time being subsequent to the elution of the reference component and identifying a time after which a desired component in the mixture will elute from the column, and
producing a signal responsive to the column output after the computed time.
14. The method as claimed in claim 13, wherein said signal is produced by integrating an electrical signal representative of the column output.
15. A method of determining the concentration of a component in the breath of a person with a gas chromatographic column comprising the steps of:
inserting a preselected reference component having a recognizable elution characteristic into a carrier gas stream coupled to the chromatographic column;
introducing a sample of the breath from a person into the carrier gas for entrainment thereby through the column;
detecting separated components emerging from the column;
measuring the elution time and the duration of the reference component;
defining in response to the measured elution time and duration a time period when there will emerge from the column a desired component the elution time and duration of which have a predetermined relationship with said reference component elution time and duration;
generating an electrical signal representative of the separated desired breath component; and
integrating the electrical signal for said defined time period.
16. A method of determining the concentration of a component in a mixture with a chromatographic column comprising the steps of:
measuring the time required for the passage of a uniquely recognizable reference component through the chromatographic column to a detecting station;
selecting a desired component emerging from the column for analysis during a time period the start time of which bears a predetermined ratio to the measured time for passage of said reference component; and
producing a measurement signal responsive to the column output during said time period.
17. A method of selecting a component in a mixture for analysis from a chromatographic column comprising the steps of:
inserting a reference component having a uniquely recognizable elution characteristic into a carrier fluid stream coupled to a chromatographic column;
introducing a sample of the substance to be analyzed into the carrier fluid stream;
detecting components separately emerging from the column;
recognizing the detected reference component characteristic;
determining the time period required for the reference component to pass through the column; and
selecting a desired separated mixture component following the reference component at a subsequent time period the start time of which is determined in preselected relationship with said determined time period for passage through the column of the reference component.
18. The method as claimed in claim 17, wherein said preselected relationship is a predetermined ratio between the passage time of the reference component and the time duration between the introduction of said
sample and the start time of said subsequent time period.

19. The method as claimed in claim 18, wherein the selecting step further includes the steps of:
measuring the time width of the reference component after passage through the column; and
selecting the desired component for analysis for a time period duration bearing a predetermined ratio to the measured time width of the reference component.

20. In chromatographic analysis of a fluid sample wherein the sample is passed through a chromatographic column to produce at the column output time-separated component-peak elutions the amplitudes of which are detected for analysis purposes;
the improved method of selecting a particular component for analysis which comprises:
controlling the input to the column at a start time to produce in the output thereof a reference peak which precedes the component to be selected and which has a unique detectable output signal characteristic distinguishing such reference peak from the peak of any other component in the sample, the time of appearance of said reference peak and of said particular component being related by a certain known function;
producing a first signal responsive to the detection of said reference peak and indicating the time between said start time and the time of detection of said reference peak;
producing a second signal bearing a predetermined relationship to said first signal in accordance with said certain known function to identify the start of a subsequent time period during which the desired component is at the column output; and
selecting for analysis the column output signal appearing during the time period identified by said second signal.

21. For use with chromatographic apparatus of the type including a column through which a fluid sample is to be passed for the purpose of separating on a time scale the components of said fluid sample for individual detection by a detecting apparatus;
that improvement for automatically selecting a particular component for analysis which comprises:
control means to control the input to the column to produce in the output a reference peak preceding the particular component to be selected and having a unique characteristic distinguishing such reference peak from the output signal produced by any other component in the fluid sample;
recognition means operable with said detecting apparatus to recognize said unique characteristic while the material is eluting from the column;
means under the control of said recognition means to produce a first signal indicative of the time of passage through said column of said reference peak;
computation means responsive to said first signal while said fluid sample continues to elute from said column and operable to produce a selection signal identifying on said time scale a subsequent time period the start of which bears a predetermined relationship to the preceding time period of detection of said reference peak; and
selection means operable during said subsequent time period to produce an output signal responsive to the output of said column while the component of interest is being detected.

22. Apparatus as set forth in claim 21, including means for producing a width signal responsive to the width of the reference peak;
said computation means including means responsive to said width signal for controlling the duration of said subsequent time period in accordance with a predetermined relationship to said width signal.

23. For use with on-line chromatographic apparatus of the type including a column through which a fluid sample is to be passed for the purpose of separating on a time scale the components of said fluid sample for individual detection by a detecting apparatus;
that improvement for automatically selecting a particular component for analysis which comprises:
control means to control the input to the column to produce in the output a reference peak preceding the particular component to be selected and having a unique characteristic distinguishing such reference peak from the output signal produced by any other component in the fluid sample;
recognition means operable with said detecting apparatus to recognize said unique characteristic while the material is eluting from the column;
means under the control of said recognition means to produce a first signal indicative of the time of passage through said column of said reference peak;
computation means responsive to said first signal while said fluid sample continues to elute from said column and operable to produce a selection signal identifying on said time scale a subsequent time period the start of which bears a predetermined relationship to the preceding time period of detection of said reference peak; and
selection means operable during said subsequent time period to produce an output signal responsive to the output of said column while the component of interest is being detected.
appearance of said desired component at the column output.  
25. The method of claim 24 carried out in an on-line chromatographic analysis wherein said first signal is produced when said reference peak is detected and said second signal is produced while portions of the sample subsequent to said reference peak are eluting from the column.  
26. In chromatographic analysis of a fluid sample wherein the sample is passed through a chromatographic column to produce at the column output time-separated component-peak elutions;  
the improved method of selecting a particular component-peak which comprises:  
controlling the input to the column at a start time to produce in the output thereof a reference peak which precedes the component peak to be selected and which has a unique detectable output signal characteristic distinguishing such reference peak from the peak of any other component in the sample, the times of appearance of said reference peak and said component peak having a predetermined relationship;  
producing a first signal responsive to the detection of said reference peak and representative of the time between said start time and the time of detection of said reference peak;  
producing a second signal responsive to said first signal and in accordance with said predetermined relationship to identify the selected component-peak signal; and  
performing in response to said second signal an operation related to said desired component.  
27. The method of claim 26, wherein said operation comprises producing a peak signal responsive to the detection of said selected component;  
zeroizing an integrator at the time of said second signal; and  
applying said peak signal to said zeroized integrator.  
28. The method of claim 26, including:  
producing a third signal representative of the time duration of said reference peak; and  
performing a mathematical operation on said third signal to compute a predicted time period following said start time and during which the desired component will be available for sensing.  
29. The method of claim 28, including the step of integrating the selected component-peak signal throughout said computed time period.  
30. The method of claim 29, including the step of zeroizing an integrator at the time of said second signal; and  
applying said selected component-peak signal to said zeroized integrator.  
31. For use with chromatographic apparatus of the type including a column through which a fluid sample is to be passed for the purpose of separating on a time scale the components of said fluid sample for individual detection by a detecting apparatus;  
that improvement for automatically selecting a particular component for analysis which comprises:  
control means to control the input to the column to produce in the output a reference peak preceding the particular component to be selected and having a unique characteristic distinguishing such reference peak from the output signal produced by another component in the fluid sample;  
recognition means operable with said detecting apparatus to recognize said unique characteristic;  
means to produce a first signal indicative of the time of passage through said column of said reference peak;  
computation means responsive to said first signal and operable therewith to produce a selection signal identifying, on said time scale, the start of a subsequent time period with the start time bearing a predetermined relationship to the time of detection of said reference peak;  
an integrator under the control of said selection signal and operable to produce an output signal corresponding to the time-integration of the selected component peak; and  
means responsive to said selection signal for zeroizing said integrator prior to the integration of said selected component peak.  
32. In chromatographic equipment of the type including a column through which a fluid sample is to be passed for the purpose of separating on a time scale component peaks of said fluid sample;  
that improvement for use in selecting a particular component comprising:  
control apparatus for controlling the input to the column to produce in the output thereof a reference peak preceding the peak of the desired component to be selected and having a unique characteristic distinguishing such reference peak from the output signal produced by any other component in the fluid sample, the times of appearance of said reference peak and the peak desired to be selected being related in accordance with a predetermined function;  
a detector responsive to the column output and arranged to recognize said unique characteristic;  
a signal-producing device coupled to said detector to produce a first signal representative of the time of appearance of said reference peak;  
computation apparatus responsive to the time represented by said first signal and operable to produce in accordance with said predetermined function a selection signal identifying the desired component; and  
an output element under the control of said selection signal to produce a function related to the appearance of said desired component.  
33. In a chromatographic process wherein a sample mixture is introduced to a chromatographic column for separation of components thereof, the method comprising the steps of:  
measuring the time required for the elution of a uniquely recognizable reference component from the chromatographic column;  
computing the start time of a time period the start of which bears a predetermined relationship to the measured reference component elution time, with the computed start time being subsequent to the elution of the reference component and identifying a time after which a particular component of the sample mixture will elute from the column;  
producing a signal indicating said computed start time; and
performing in response to said signal a function related to the appearance of said particular component.