

J. M. Gross,¹ B.Sc. and L. J. Porter,¹ M.Sc.

Automatic Reduction of Multicolumn Chromatographic Data

An approach is given using the Perkin-Elmer PEP-2 chromatographic Data System [1] to combine data from both columns in a gas chromatograph. The Interactive Programming Module (IPM) option [2] is required. Program language is Interactive Programming Language (IPL). The component of interest is not always separated adequately from interfering substances in a single column analysis. Thus, a sample is frequently analyzed under more than one set of chromatographic conditions. The component of interest must be identified on each column to give a positive result. When sample volume is large it is convenient to have on-line data processing, such as the PEP-2 dedicated system. A printout of computed results from each column is quantitatively superior to manual chromatogram evaluation techniques. Combining all results for a sample in one report is the approach applied by the authors to a dual column configuration described by Adams [3]. If a drug is identified on both columns, further confirming analyses are pursued.

Procedure

For simplicity, the program developed here will be limited to three components, amphetamine (*AM*), secobarbital (*SE*), and methaqualone (*MQ*), with barbital (*BA*) as reference peak. A mixture of the three components was analyzed on a Perkin-Elmer Model 3920 dual column gas chromatograph equipped with capsule injection and sample splitter at the inlet. The two chromatographic channels will be designated instrument-1 (OV-17) and instrument-2 (OV-1). The component and instrument from which results are obtained are indicated as *AM1*, *AM2*, and so on. A method of analysis contains information required by the PEP system to prepare a report. Included in a method are reference peak retention time, relative retention times, response factors, and names of peaks for identification. Response factors are determined experimentally and are related to extraction efficiency and detector response. This definition applies to "method" throughout this paper. Methods must yield data from both instruments in the same units of concentration. The analyst may choose his own units of concentration. With appropriate response factors both instruments should provide equivalent results. Practically, this occurs only when both instruments show results of zero.

The actual IPL program using the above principle for three components, amphetamine, secobarbital, and methaqualone, consists of the parts listed in Table 1. Analytical definition statements specify method, instrument, sequence, and item numbers. The sequence number is 1 unless the same instrument is used twice. Examples of this

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¹Principle chemist and project director, respectively, Alcohol/Drug Highway Safety Program, Ohio Department of Health, Columbus, Ohio 43216.

TABLE 1—*The IPL program for three components.*

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1. Program generate directive
 2. Analytical definition statements
 3. Numeric definition statements
 4. Algebraic statements
 5. Concentration control statements
 6. Print statements
 7. End statement
-

are changing the column and injecting the sample in the same instrument or changing conditions for a different component. In either case a different method would be needed. Item number is the order of elution for each peak identified by the designated method. Numeric definition statements give initial values to each symbol not found in an analytical definition. The concentration control statement causes the value of a symbol to be stored in the concentration register. A print statement causes the value currently stored in the concentration register to be printed after a text of up to 60 characters.

Secobarbital is separated insufficiently from an interfering substance (caffeine, *CA*) on instrument-2. Quantitative results from instrument-2 are not wholly reliable in this case, as shown by comparative chromatograms in Fig. 1. Peak overlap where Peak 2 is larger than Peak 1 prevents the use of PEP-2 Data System "tangential skim threshold" for quantitative resolution. The quantitation will therefore be based on instrument-1 only. Secobarbital must still be identified by instrument-2 to give a positive result in this approach to data reduction.

The calculation program to be entered in the PEP-2 Data System is as shown in Fig. 2. Line numbers listed at the far left are not part of the program, but are used to identify explanations. The numbers in the center column are not part of the program but are references to the statement types listed in Table 1. Line 1 signals the computer that program number two is being generated. Lines 2 through 7 give analytical definitions for *AM1*, *AM2*, *SE1*, *SE2*, *MQ1*, and *MQ2*. Lines 8 and 9 give initial values to symbols other than those representing peaks, and *K* remains constant throughout the program. Line 10 gives instructions for calculation of *H* for amphetamine. The asterisk means multiply. Line 11 places the value of *H* in the concentration register. Line 12 causes the word "amphetamine" to be printed followed by the value stored in the concentration register. One blank space is left after "amphetamine" to cause the value to be aligned with the values of *SE* and *ME*. Notice the different equation used in Line 13 for *SE*, which may not be totally resolved from an interference in instrument-2. Line 19, two colons, signals the computer that the program is concluded.

An example of the program output is shown in Fig. 3. The first line is automatically printed in all program outputs by the PEP-2 Data System and has no relevance to this program.

Discussion

If the component of interest is amphetamine, *AM1* and *AM2* are the concentrations obtained from instrument-1 and instrument-2, respectively. The arithmetic mean *A* is frequently used as the reported value. This is satisfactory if amphetamine is actually present. If it is absent but an interference is identified as amphetamine on instrument-1, the result would be

$$A = (AM1 + 0)/2$$

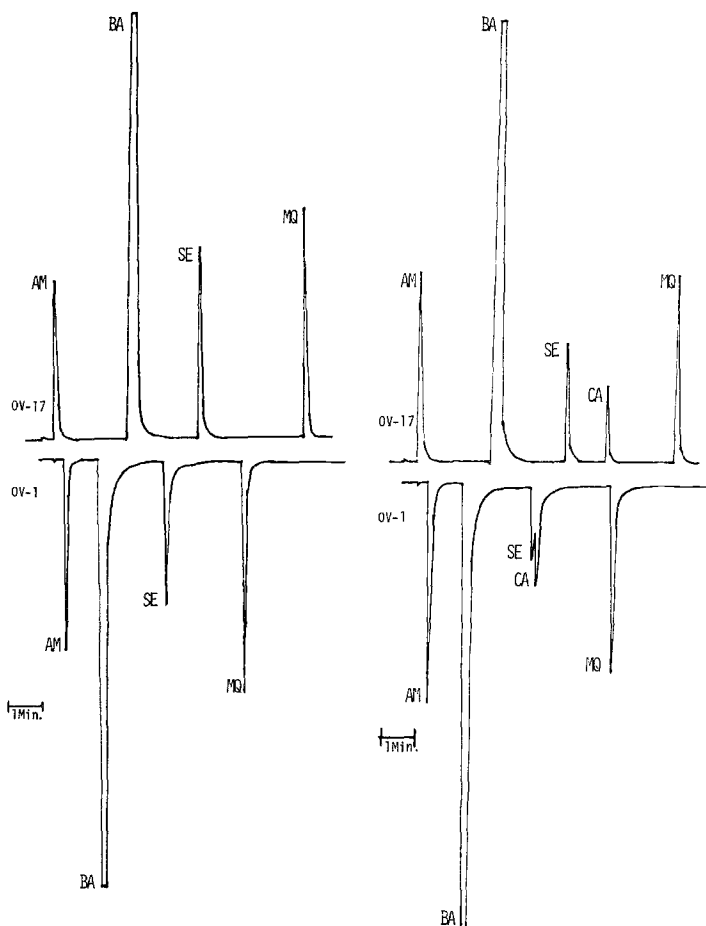


FIG. 1—Chromatograms depicting secobarbital SE peaks with and without caffeine CA present as an interfering component on OV-1 column. Analytical procedure by Adams [3].

This situation is eliminated by the program. One solution is to multiply $AM1$ by $AM2$. If either is zero the product is zero. However, if amphetamine is present, the multiplication will give the square of the concentration. In IPL the allowed mathematical operations are add, subtract, multiply, and divide. Since square roots, "if" statements, and "loops" are not available in IPL, a geometric mean G cannot be used. The harmonic mean H is the square of the geometric mean G^2 divided by the arithmetic mean A . Equation 1 gives H for AM .

$$H = (2AM1 AM2)/(AM1 + AM2) \quad (1)$$

In the case where $AM1$ and $AM2$ are equal and not zero, H is given by

$$H = (2AM^2/2AM) = AM$$

In this case H is equal to A . If $AM1$ and $AM2$ are not equal, H is less than A . The relation between H and A is given by

$$H = A - [(AM1 - AM2)^2/2(AM1 + AM2)]$$

Line	Statement Type	
1	1	/PG.2:
2	2	AM1.(METH11,INST1,SEQ 1,ITEM1:
3	2	AM2.(METH21,INST2,SEQ 1,ITEM1:
4	2	SE1.(METH11,INST1,SEQ 1,ITEM3:
5	2	SE2.(METH21,INST2,SEQ 1,ITEM3:
6	2	MQ1.(METH11,INST1,SEQ 1,ITEM4:
7	2	MQ2.(METH21,INST2,SEQ 1,ITEM4:
8	3	H.;0:
9	3	K.;.0001:
10	4	H.=2,*AM1.*AM2./(AM1.+AM2.+K.):
11	5	CØNC.H.
12	6	P.AMPHETAMINE :
13	4	H.=SE1.*SE2./(SE2.+K.):
14	5	CØNC.H.
15	6	P.SECØBARBITAL:
16	4	H.=2,*MQ1.*MQ2./(MQ1.+MQ2.+K.):
17	5	CØNC.H.
18	6	P.METHAQUALØNE:
19	7	::

FIG. 2—The calculation program to be entered in the PEP-2 Data System.

INST	TYPE	FILE	METH	C	NAME
	AMPHETAMINE		2.3105,		
	SECØBARBITAL		2.4357,		
	METHAQUALØNE		1.0780,		

FIG. 3—An example of the program output.

In the PEP-2 Data System, division by zero causes the program to be deleted; therefore, Eq 1 cannot be used. To avoid a value of zero in the denominator, a small constant K is added to the sum of $AM1$ and $AM2$. With K , we now have Eq 2.

$$H' = (2AM1 AM2)/(AM1 + AM2 + K) \quad (2)$$

If the values of AM are large compared to K , the effect of adding K to the denominator is minimal. The smallest and largest values greater than zero which can be handled by the PEP-2 Data System input/output are 0.0001 and 32 767, respectively (32 767 is one less than 2^{15}).

Table 2 gives values of A , H' , and H for values of $AM1$ and $AM2$ corresponding to arbitrary units to demonstrate the range of usefulness. In calculating H' the value of K was 0.0001. Notice that close agreement for values of $AM1$ and $AM2$ yield values of A and H' which are in close agreement. The values of $AM1$, $AM2$, and H' were calculated by the PEP-2 and have four significant figures. To take advantage of the four significant figures available for component concentrations, units should be chosen such that the smallest expected value of concentration is 0.1000. This precision is greater than reproducibility in gas chromatography.

TABLE 2—Values of A , H' , and H for values of $AM1$ and $AM2$ corresponding to arbitrary units.

$AM1$	$AM2$	A	H'	H
2.0002	0.0000	1.0001	0.0000	0.0000
0.0010	0.0009	0.0009	0.0009	0.0009
0.0684	0.0629	0.0656	0.0655	0.0655
1.0001	0.8868	0.9434	0.9400	0.9400
17.3829	15.9817	16.6823	16.6528	16.6529
325.9221	299.6514	312.7867	312.2350	312.2351
20001.7504	17735.6960	18868.7232	18800.6861	18800.6873

This principle can be extended to the case of three chromatographic instruments. The concentrations from the three instruments of component X are designated X_1 , X_2 , and X_3 . Two divisions are required in this case. The quotient of the first division Q_1 is given by

$$Q_1 = (9X_1X_2X_3)/(X_1 + X_2 + X_3 + K)$$

The desired concentration of X is given by

$$X = Q_1/(X_1 + X_2 + X_3 + K)$$

Summary

A program technique for reducing multicolumn chromatographic data was developed which consolidates independent quantitative results into a single report. A Perkin-Elmer PEP-2 Data System equipped with the interactive program module was used. The harmonic mean in this calculation program enables the user to be presented a negative result where the component is positive on only one chromatographic column. Variance between arithmetic mean and harmonic mean in a properly matched dual column system is minimal. This application is useful with programmable processors which have neither square root function nor "if" statements.

References

- [1] "Pep-2 Data System Instructions," Perkin-Elmer Corp., Norwalk, Conn., 1974.
- [2] "Interactive Programming Module Instructions," Perkin-Elmer Corp., Norwalk, Conn., 1975.
- [3] Adams, R. F., "Drug Analysis by Simultaneous Dual-Column GLC," *Clinical Chemistry Newsletter*, Vol. 4, No. 1, 1972, pp. 15-28.

Alcohol/Drug Highway Safety Program
Ohio Department of Health
450 E. Town St.
P.O. Box 118
Columbus, Ohio 43216