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Application of the Microcomputer to Analytical Toxicology

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ABSTRACT: The recent availability of the microcomputer, marketed primarily for home use, has given the analytical toxicologist a very inexpensive source of computer capability. Probative studies have shown that the microcomputer can be applied to statistical analysis, to data base searching, and to calculations of the discriminating power of analytical systems.

KEYWORDS: toxicology, computers

Computers and microprocessors are an integral part of the modern forensic science laboratory. These units collect and process analytical data, control instruments, make complex statistical calculations, and store scientific and administrative information. Laboratories equipped with minicomputers[2] generally have self-contained laboratory automation systems consisting of hardware and dedicated software. Such systems are primarily designed for sophisticated peak measurement, integration, and calculation of chromatographic or spectrophotometric analyses. Optional memory expansion to carry out individualized BASIC programs is available but expensive.

There are some reports in the toxicology literature of the general application of minicomputers. In 1974, Kazyak [*1*] described a retrieval system using a minicomputer for analytical data and literature references. Another report by the Bureau of Forensic Sciences [*2*] describes a system of two minicomputers serving many laboratories for qualitative searches on analytical data. A sophisticated application for forensic science has been also reported by Pearson and Brown [*3*].

In the field of toxicology, larger computer systems have been primarily applied to literature retrieval and to information sources for poison control centers [*4,5*]. A description of toxicology resources available from commercial computer services is discussed by Dessy and Starling [*6*]. These applications were primarily accomplished with minicomputers.

The recent introduction of microcomputers marketed for "personal home use" has resulted in a major cost reduction in computer use. A 32K memory microcomputer with screen (cathode-ray tube), keyboard, and cassette drive is available from about $1300.

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[2] For the purpose of this report, "minicomputer" refers to those units usually costing more than $30 000 and "microcomputer" refers to the units marketed as personal home computers and costing less than $1500.

Examples of microcomputers are the Apple II (Apple Computer, Inc.), the TRS 80 (Tandy Corp.), and the PET 2001 (Commodore Business Machines). All the microcomputers use BASIC language but have different cassette tape input systems. (This means that a cassette data/program is not interchangeable from one manufacturer's unit to another.) Introductory information regarding microcomputers and BASIC software programs is available from computer salespersons and retail shops as well as literature sources [7-9]. An abundance of software programs for microcomputers exists for games, statistical calculations, inventory control, and various business techniques. However, little has been published in regard to applications of the microcomputer for the scientist and, in particular, the toxicologist.

Some uses of the relatively inexpensive microcomputer were evaluated in a toxicology section. The projects considered in this evaluation were routine statistical calculations, searches of analytical data bases, and replicate comparisons of data to calculate the discrimination between analytical systems.

Equipment and Operation

The microcomputer used in this study was the Commodore PET 2001, consisting of a cathode-ray tube (CRT) display, a keyboard, and a cassette tape drive integrated into a compact desk-top unit. There was 14K of memory used for a BASIC interpreter operating built-in programs and peripheral utility programs. The random access memory (RAM) occupied 8K address and was expanded to 32K by the purchase of additional memory. The unit was equipped with an Institute of Electrical and Electronic Engineers (IEEE) Standard 488 bus for programmable instruments and cost about $1300. An Integrated Data Systems Model DMTP-6 printer (cost about $1000) was useful for recording data entry and results and for listing the programs for study during the early stages of programming.

The program preparation was done by one of the authors (B. P.), who learned sufficient BASIC language from a text [*10*] to develop the program described. The programs discussed in this report were originally prepared and stored on cassette tape.[3] Subsequent operation of the program is effected by loading the tape and starting the program.

Applications

Statistical Analysis

The application of statistics to quantitative toxicological analysis is common. Although many scientific calculators contain a number of hard-wired commands to give useful statistics, the data entered cannot be readily checked nor the calculations easily shown without the use of a printer. A program for linear regression analysis was prepared that allowed a check of entry data (such as concentration and peak ratio response for preparation of a calibration graph) and clearly showed the statistics (slope, intercept, standard deviation estimate, and correlation coefficient).

The program shown in Fig. 1 illustrates the ease with which complicated statistical programs can be written in BASIC language. The capability of reviewing the data input prior to the calculation and of viewing all the statistics on the screen is of practical advantage.

Other statistical programs that have been developed are the calculation of standard deviation and χ^2 analysis for gene frequency calculations in serology studies. The advantage of applying the microcomputer to these calculations is that the programs can be developed for specific in-house requirements to give instantaneous results.

[3] A Diskamon dual disk drive unit (approximate cost $1500) has been added to the system, thus enlarging memory and decreasing the program loading time considerably. A successful index system for toxicology literature has been developed for title, author, source, and keyword searches of collected papers.

```
                  PROGRAM                                  (REMARKS)

10 INPUT" NUMBER OF DATA POINTS ";N                       : REM - SAMPLE SIZE.
20 DIM X(N),Y(N)                                          : REM ----------------
30 FOR K = 1 TO N                                         : REM - INPUT AND
40 INPUT" X VALUE ";X(K)                                  : REM - STORAGE OF
50 INPUT" Y VALUE ";Y(K)                                  : REM - 'N' X,Y
60 NEXT K                                                 : REM - POINTS.
70 FOR K = 1 TO N                                         : REM ----------------
80 PRINT K"   "X(K)"     "Y(K)                            : REM -
90 NEXT K                                                 : REM - PRINT OUT OF
100 INPUT" ARE VALUES CORRECT? (Y OR N)";Q$               : REM - DATA AND
110 IF Q$ = "Y" THEN GOTO 160                             : REM - CORRECTION OF
120 INPUT" WHICH NUMBER IS WRONG?";K                      : REM - ANY INPUT
130 INPUT" X VALUE ";X(K)                                 : REM - ERRORS.
140 INPUT" Y VALUE ";Y(K)                                 : REM -
150 GOTO 70                                               : REM ----------------
160 FOR K = 1 TO N                                        : REM -
170 X = X + X(K)                                          : REM - ADDITION OF
180 Y = Y + Y(K)                                          : REM - REQUIRED
190 X2  =  X2 + X(K)↑2                                    : REM - STATISTICAL
200 Y2  =  Y2 + Y(K)↑2                                    : REM - SUMS.
210 XY  =  XY + X(K)*Y(K)                                 : REM -
220 NEXT K                                                : REM ----------------
230 B = (N*XY-X*Y)/(N*X2-X*X)                             : REM - SLOPE.
240 A = (Y-B*X)/N                                         : REM - INTERCEPT.
250 R = (N*XY-X*Y)/(((N*X2-X*X)*(N*Y2-Y*Y))↑.5)           : REM - CORR. COEFF.
260 S = ((Y2-(A*Y+B*XY))/(N-2))↑.5                        : REM - SD(Y.X).
270 PRINTB,A,R,S                                          : REM -  PRINT OUT.
280 END                                                   : REM ----------------
READY.
```

FIG. 1—*Linear regression program useful for quantitative procedures.*

Analytical Data Base Searching

A large sample of data of the more commonly encountered basic and neutral compounds was used as a base for the development of a search procedure. An example of the data base is shown in Fig. 2. The data incorporated and loaded for 177 compounds included:

(1) for gas chromatography (GC), the retention indices (using temperature programming) on SE-30, OV-7, and OV-17 columns;

(2) for thin-layer chromatography (TLC), the $R_f \times 100$ values for the common systems methanol/ammonia (100:1.5) (T1) and ethylacetate/methanol/ammonia (17:2:1) (ET) as well as the visualization and characteristics after the spray sequence: ultraviolet (U), fluram spray (F), ninhydrin spray (N), sulfuric acid spray (H), ultraviolet (U), and potassium iodoplatinate spray (I);

(3) for ultraviolet (UV) spectrophotometry, the maximum wavelength in sulfuric acid (UVA) and in sodium hydroxide (UVB) with the respective extinction coefficients (A1%);

(4) the molecular weight (MWT); and

(5) the base peak value (BPK).

The data base and search program are entered into the microcomputer and stored on a cassette tape. The tape is loaded only once into the computer to do searches. The data base can be searched for data from GC, TLC, UV, or base peak entries. Search windows can be applied to add flexibility to the search. (For example, a TLC $R_f \times 100$ value of 45 used with a window of ± 3 would select all the compounds with R_f from 42 to 48.)

An example of a search of the data base is shown in Fig. 3. The example assumes the

D#	SE30	OV7	OV17	UVA	A1%	UVB	A1%	T1	ET	UFNHUI	BPK	MWT	NAME
D359	2425	2840	3070	280	031	---	---	53	--	*****X	---	315	OXYCODONE
D360	2170	2510	2700	279	66	301	278	14	23	-PBWPX	245	260	OXYMETAZOLINE
D374	2285	2460	2620	278	112	298	67	60	72	-P---X	70	285	PENTAZOCINE
D376	2200	----	2730	253	850	255	202	58	43	PGY-PX	42	404	PERPHENAZINE
D377	1740	1870	2000	257	9	257	7	48	65	-P---X	71	247	PETHIDINE
D383	1890	2000	2140	263	12	---	---	59	80	--PWPX	200	243	PHENCYCLIDINE
D384	1445	1555	1670	261	13	---	---	50	64	-----X	57	191	PHENDIMETRAZINE
D386	1120	1220	1310	258	21	257	---	33	35	-GRROX	91	121	PHENETHYLAMINE
D389	1805	1955	2105	261	318	261	208	32	59	*****X	---	240	PHENIRAMINE

FIG. 2—*A portion of the 177-compound data base used for the study of analytical data base searching. The first column* (D#) *shows code numbers for inventory purposes.*

initial screen of the basic fraction of a liver yielded a GC retention index of 2190 with an SE-30 column (temperature program) and an R_f ($\times 100$) value of 50 using the TLC T1 system. The search of the data base was initiated with the SE-30 value using an error factor of ± 10. (This is a narrow window but is quite acceptable for temperature program analysis.) The computer search found six compounds in the data base with a retention index of 2180 to 2200. A qualifying search of these six compounds using the TLC datum of 50 ± 3 (T1 system) found two compounds that fit both searches. The data for the two compounds were called up by the code numbers (D#'s), which yielded a complete data description. The qualifying searches can be continued with any other analytical parameters and in any order. The search is done almost as fast as the case data can be typed.

The search program described here was confined to 177 compounds to evaluate the feasibility of analytical data searching. The number of compounds and data can be increased to accommodate a larger data base. Besides the advantage of faster searching (than by using GC, UV, and TLC tables), the computerized data base allows the flexibility of using search windows, thus increasing the scope of the total search.

Calculation of Discriminating Power

Discriminating power (*DP*) has been defined as the probability that two compounds selected at random would be distinguished from each other in that system *[11]*. Such calculations have been suggested as a means to initially judge whether one analytical system is more selective (or better) than another analytical system or combinations of analytical systems (such as GC and TLC) *[12,13]*. This may eliminate the reproduction of analytical data that is usually necessary before evaluation can occur. These calculations have been useful in judging the qualitative (screening) capability of reported procedures for analytical toxicology.

Moffat et al *[11]* used a program in FORTRAN language on a minicomputer. To determine the feasibility of the microcomputer for the calculation of *DP*, a similar program in BASIC was prepared and applied to the data base of 177 compounds. In comparisons among the GC retention values on an SE-30 column with a search window of 25, the *DP* value was calculated as 0.9644. (The search window or error factor is determined by the operator to define the range of the search in the retention data base.)

The *DP* value of 0.9644 for the SE-30 column can be compared with *DP* values for other systems (such as OV-1 and OV-17). The *DP* value closest to 1 is indicative of the more selective analytical system. The *DP* values can be determined for multicomponent systems of GC, UV, and TLC.

```
SEARCH SYSTEM ?                                        SE30
  SEARCH VALUE ?                                       2190
    ERROR FACTOR ?                                       10

FOR A SEARCH OF SE30 AT 2190, EF=10
# MATCHES = 6

DO YOU WANT A PRINT OUT OF THE CODE # FOR DATA
Y, N, FIN ?                                               N

DO YOU WISH A NEW SEARCH, TO QUALIFY, OR
DATA PRINT OF CODE #

INPUT S OR Q OR D OR FIN ?                                Q

QUALIFY SEARCH
SEARCH SYSTEM ?                                          T1
  SEARCH VALUE ?                                         50
    ERROR FACTOR ?                                        3

ABOVE DATA QUALIFIED USING COL T1, SEARCH VALUE 50
EF=3 # MATCHES=2

DO YOU WANT A PRINT OUT OF CODE #
Y, N, FIN ?                                               Y
D24, D299

INPUT COMPOUND # OF INTEREST, FIN WHEN DONE
?                                              D24,   D299

D24 AMITRIPTYLINE                      D299 THONZYLAMINE

  SE30 - 2190                            SE30 - 2200
  OV7  - 2365                            OV7  - 2405
  OV17 - 2520                            OV17 - 2585
  UVAC -  240                            UVAC -  235
  A1%  -  500                            A1%  -  902
  UVB  -  242                            UVB  -  241
  A1%  -  185                            A1%  -  767
  T1   -   49                            T1   -   47
  ET   -   75                            ET   -    0
  BPK  -   58                            BPK  -  121
  MWT  -  217                            MWT  -  286

  TLC COLORS                             TLC COLORS
  UFNHUI                                 UFNHUI
  PPB-PX                                 *****X
```

FIG. 3—*A typical search to identify a compound after screening by GC and TLC.*

Conclusion

These limited probative studies have shown that the inexpensive microcomputer can be useful in the analytical toxicology laboratory. Successful areas of application were these:

(1) specific statistical calculations, such as the preparation of standard calibration graphs in quantitative procedures;
(2) searching various analytical data bases; and
(3) complex comparisons for determining the discriminating power of various analytical systems used in toxicological analysis.

The "home" microcomputer can be a definite asset to the toxicology laboratory and proves an inexpensive source (less than $2000) of computer capability.

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