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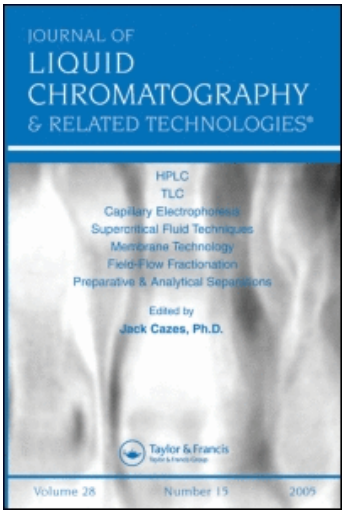


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Simultaneous Multi-Wavelength UV-Visible Detection for HPLC Using a Diode-Array Spectrophotometer—A Versatile Software Package

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**To cite this Article** Iwaniw, Diana C. and Josephy, P. David(1985) 'Simultaneous Multi-Wavelength UV-Visible Detection for HPLC Using a Diode-Array Spectrophotometer—A Versatile Software Package', Journal of Liquid Chromatography & Related Technologies, 8: 2, 387 — 394
**To link to this Article: DOI:** 10.1080/01483918508067086
**URL:** http://dx.doi.org/10.1080/01483918508067086

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JOURNAL OF LIQUID CHROMATOGRAPHY, 8(2), 387-394 (1985)

# SIMULTANEOUS MULTI-WAVELENGTH UV-VISIBLE DETECTION FOR HPLC USING A DIODE-ARRAY SPECTROPHOTOMETER—A VERSATILE SOFTWARE PACKAGE

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ABSTRACT

A BASIC program was developed to monitor absorbance simultaneously at various wavelengths using an HP8451A Diode Array Spectrophotometer linked to a HPLC system and equipped with a flow cell. The program measures a maximum of ten wavelengths and takes a maximum of 240 measurements for each wavelength, using only 16K of memory and a dual disc drive. The program includes the options of replotting using a different ordinate scale and saving the numerical data on disc.

## INTRODUCTION

In conventional HPLC detection using a single wavelength detector, compounds not absorbing at the chosen wavelength may be missed. In the system described here, the HPLC is linked to a

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diode-array spectrophotometer using a flow cell. A BASIC program was developed to perform the necessary operations to adapt the spectrophotometer for use as a multiwavelength detector.

## EQUIPMENT

Our laboratory set up comprises a HP 8451A Diode Array Spectrophotometer, HP 98155A keyboard, HP 9121 Dual Disc Drive, HP 7470A Plotter, Waters Z-module Radial Compression System with a C18 column, two Waters Model S10 pumps, Waters U6K injector and Waters Automatic Gradient Controller. The spectrophotometer was equipped with a HP 89070A adjustable Cell holder and a Hellma 8 µl flow cell. The system also included a Waters Model 440 single wavelength detector and a HP 3392A integrator, used to monitor absorbance at 254 nm during the run.

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## PROGRAM DEVELOPMENT

The object of the program was simultaneous measurement of absorbance at multiple wavelengths. The option of replotting the chromatogram using an alternate ordinate scale requires storage of data on disc. A second disc is used for data storage to avoid shortage of space on the main program disc. The program is divided into two parts to make efficient use of available memory. The first part measures and stores data, while the second recalls and plots data. The two parts link automatically and run as a single program.

The option of saving the data in numerical form requires changing the data file name (RENAME DATA to "XYZ") before the program is rerun. If this is not done, the program will try to

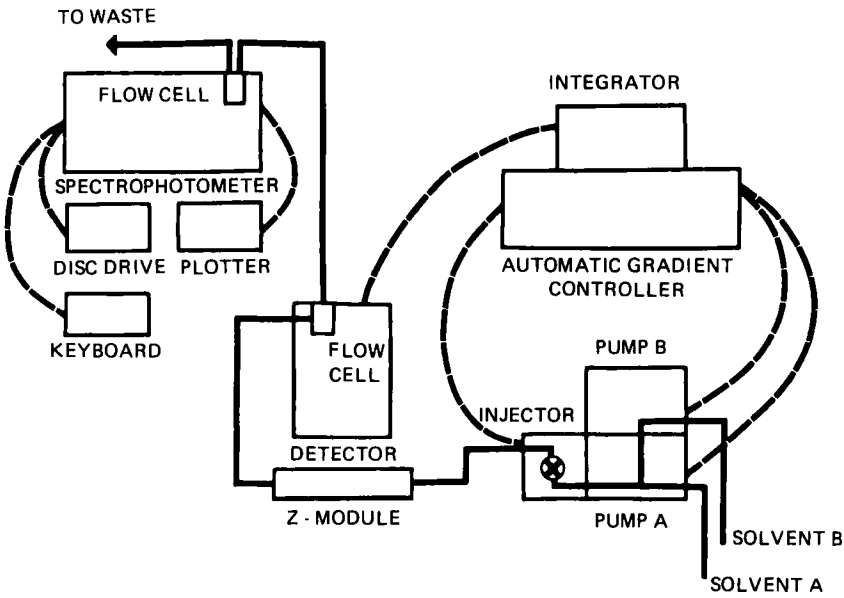


FIGURE 1

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create a file ("DATA") which already exists, and will halt. The stored data can be read using the simple program "READDATA", a listing of which is included at the end of this paper. There is no simple way of replotting the data once the program "WLGTHPLOT' is terminated.

The major limitation of the program is the number of measurement results which can be stored in memory. This number is 240 measurements for each of 10 wavelengths (with 16K of memory). This dimension can be increased if more K of memory are available. Statement 220 of program "WLGTHMEAS" should also show a corresponding increase in the number of records.

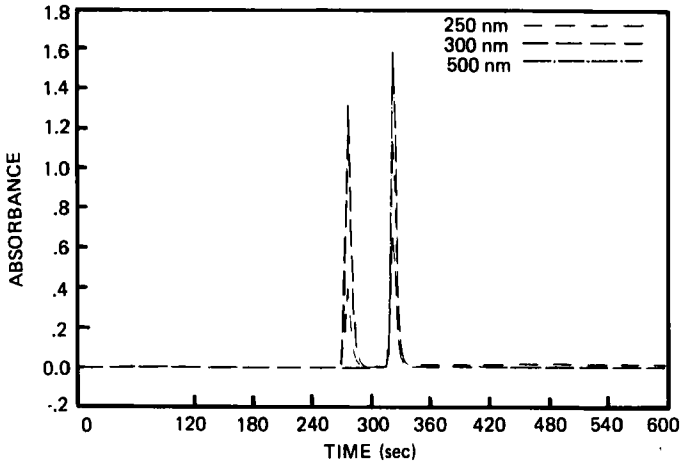


FIGURE 2

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The minimum time interval between measurements for any number of wavelengths is 0.7 seconds. Two pens of different colour must be used if the number of wavelengths exceeds five.

## EXAMPLE OF APPLICATION

A mixture of benzidine and Congo red was separated using a solvent system of 90% methanol, 10% 10 mM sodium phosphate buffer, pH 7 (A) and 10% methanol, 90% buffer (B) and the following gradient program for the Waters Automatic Gradient Controller.

| Time (min.) | Flow (ml/min) | % A | % B | Curve |
|---|---|---|---|---|
| 0 | 3.00 | 25 | 75 | * |
| 5.00 | 3.00 | 100 | 0 | 6 |

Benzidine has a $\lambda_{max}$ below 300 nm while Congo red has a $\lambda_{max}$ over 300 nm. The monitoring wavelengths chosen were 250, 300 and

500 nm. The first peak absorbed at 250 nm, and 300 nm. The second peak absorbed at all 3 wavelengths. The first peak was identified as benzidine and the second as Congo red.

[A copy of this software package is available upon request; please provide a Hewlett Packard 3-1/2" flexible disc.]

## ACKNOWLEDGEMENTS

We wish to thank the National Sciences and Engineering Research Council of Canada, the National Cancer Institute of Canada, and the Banting Medical Research Foundation (Toronto) for financial support. D.C. Iwaniw is the recipient of a $(GWC)^2$ summer research assistantship for 1984.

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"WLGTHMEAS"

```
10 ERASE STATUS                          Frees all available
20 ERASE STANDARD                        memory space
30 MASS STORAGE IS ":D701"               Access new disc
40 COM L(10),X,Y,T,S                     Dimensions and initializes
50 DIM M(10,240)                         variables
60 MODE 0,1                              Suppresses screen display
70 DISP "INPUT # OF WAVELENGTHS"

80 INPUT X                               Reads inputted wavelengths
90 DISP "INPUT WAVELENGTHS"
100 FOR N=1 TO X
110 INPUT L(N)
120 NEXT N
130 ABSORBANCE                           Determines function


140 DISP "INPUT INTEGRATION TIME"
150 INPUT R
160 DISP "INPUT MEASUREMENT              Sets up measurement parameters
          INTERVAL"
170 INPUT S
180 DISP "INPUT TOTAL TIME"
190 INPUT T
200 DISP "INPUT STARTING TIME"
210 INPUT Y
```

```
220 CREATE "DATA", 100                Creates data file and opens
230 ASSIGN# 1 TO "DATA"               buffer
240 DISP "TO REFERENCE
         HIT ENDLINE"                 References
250 INPUT A$

260 REFERENCE
270 IF NMEAS<1 THEN 270
280 DISP "MEASUREENT WILL BEGIN
          IF YOU HIT ENDLINE"
290 INPUT B$
300 MEASURE R,S,O,T
310 FOR I=1 TO INT(T/S)               Measures
320 IF NMEAS<1 THEN 320
330 FOR J=1 TO X
340 M(J,I)=VALUE(L(J))
350 PRINT# 1 ; M(J,I)                 Prints to Data File
360 NEXT J
370 DISP I
380 NEXT I
390 ASSIGN# 1 TO *                    Closes Buffer

400 MASS STORAGE IS ":D700"           Accesses main disc, loads
410 CHAIN "WLGTHPLOT"                 and executes next program
420 END
 22690
```

"WLGTHPLOT"

```
10 COM L(10),X,Y,T,S                  Carries over & dimensions
20 DIM M(10,240)                      important variables
30 DISP "TO CONTINUE INPUT 1
         TO END PROGRAM INPUT 2"
40 INPUT V                            Presents option to quit or
50 IF V=1 THEN 70                     continue program
60 IF V=2 THEN 780
70 MASS STORAGE IS ":D701"            Accesses disc 2 and opens buffer
80 ASSIGN# 2 TO "DATA"
90 A=-1.E100                          Initializes variables for
100 B=1.E100                          max/min search
110 FOR I=1 TO INT(T/S)
120 FOR J=1 TO X
130 READ# 2 ; M(J,I)                  Reads from data file

140 DISP M(J,I)
150 A=MAX(M(J,I),A)
160 B=MIN(M(J,I),B)                   Performs max/min search
170 NEXT J
180 NEXT I
```

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```
190 ASSIGN# 2 TO *                          Closes buffer
200 DISP "SET UP PENS AND PAPER"            Prompts plotter set-up

210 DISP "MAX. O.D. IS ",A
220 DISP "MIN. O.D. IS ",B
230 DISP "INPUT O.D.LIMITS"
240 DISP "MAXIMUM"                          Sets ordinate
250 INPUT C
260 DISP "MINIMUM"
270 INPUT D

280 PLOTTER 705
290 PEN 1                                   Sets plotting areas
300 FRAME
310 SCALE Y,T+Y,D,C                         Centers labels
320 LORG 6
330 DISP "INPUT # DECIMAL PLACES
          FOR X-AXIS"
340 INPUT P
350 FXD P
360 LAXES T/10,0,Y,D,1,0                    Labels axes
370 DISP "INPUT # DECIMAL PLACES            numerically allowing
          FOR Y-AXIS"                       user to input no.
380 INPUT G                                 of digits in labels
390 FXD G
400 LAXES 0, (ABS(C)+ABS(D))/10,Y,C,0,1
410 MOVE Y-T/10,(ABS(C)-ABS(D))/2
420 DEG
430 LDIR 90
440 LABEL "ABSORBANCE"                              Labels axes
450 MOVE (T+2*Y)/2,D-(ABS(C)+ABS(D))/10             with titles
460 LDIR 0
470 LABEL "TIME (sec)"
480 L=3
490 PEN 1
500 FOR J=1 TO X
510 LINETYPE L+J                            Sets linetype
520 A=0
530 FOR I=1 TO INT(T/S)                     Plots data vs. time
540 PLOT Y+A*S,M(J,I)
550 A=A+1
560 NEXT I
570 PENUP
580 IF J<5 THEN 610
590 L=-2
600 PEN 2
610 NEXT J
620 PEN 1
630 LOCATE 100,180,10,110
640 SCALE 0,10,-12,2
650 F=3
660 FOR J=1 TO X
```

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"WLGTHPLOT" (CONT'D)

```
670 MOVE 0,-(H/2)                          Draws a legend
680 LABEL L(H),"nm"
690 LINETYPE H+F
700 MOVE 1,-(H/2)
710 IDRAW 3,0
720 IF H<5 THEN 750
730 F=-2
740 PEN 2
750 NEXT H
760 PEN 0
770 IF V=1 THEN 30
780 DISP "TO KEEP FILE INPUT 1
          TO ERASE FILE INPUT 0"           Presents option to store or
790 INPUT Q                                erase data


800 IF Q=1 THEN 830
810 PURGE "DATA"                           Erases data
820 MODE 4
830 MASS STORAGE IS ":D700"                Accesses main disc
840 END
 424
```

"READ DATA"

```
10 ASSIGN# 2 TO "FILENAME"                 Opens buffer
20 FOR I=1 TO (# MEASUREMENTS)
30 FOR J=1 TO (# WAVELENGTHS)              Reads and displays data
40 READ# 2; M(J,I)
50 PRINT M(J,I)
60 NEXT J
70 NEXT I
80 ASSIGN# 2 TO *                          Closes buffer
90 END
```

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