UV INSTRUMENTS AT WORK

Applications of UV-Visible Derivative Spectrophotometry

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Part II Some potential areas of application of UV-Visible derivative spectroscopic techniques.

Introduction

In part I, after a very brief historical introduction, an overview of the areas of application was presented, and the basic principles of the derivative technique were discussed in some detail.

The accelerating interest in the use of derivative techniques in UV-Visible spectroscopic analyses is further illustrated by the additional number of recently published papers, some of which are listed here.

The major part of this paper will be devoted to illustrating the power and usefulness of the derivative technique in various areas of UV-Visible spectroscopic measurements.

The various topics in Part II are discussed under the following headings:

- Very Recent Areas of Application
  - Clinical-Pharmaceutical-Biochemical (Life Sciences)
  - Inorganic
  - Miscellaneous
- Experimental
- Measurements and Discussion
  - Characterization of Individual Pure Compounds
  - Study of Homologous and Isomeric Series of Compounds
  - Quantitative Determination of Trace Components
  - Minimization and Elimination of Background Absorption
  - Characterization of Commercial Materials and Natural Substances
- Conclusion

Very Recent Areas of Application

In this, again by no means complete, selection of papers published in the last few years the increasing recognition of the usefulness of derivative techniques is further re-inforced, particularly in the so-called 'Life Sciences' area, where analyses most often have to be carried out under adverse conditions, i.e. in the presence of strongly interfering (absorbing and scattering) background matrices.

Clinical-Pharmaceutical-Biochemical (Life Sciences)

Analysis of colouring agents in pharmaceuticals by derivative ultraviolet-visible spectroscopy. [122]

Determination of morphine and heroin by second derivative UV-Spectrophotometry. [123]

Pharmaceutical applications of computer-aided optical multi-channel Spectroscopy. [124]

Stability of oral vitamin K — a comparison of an HPLC and derivative spectrophotometric method. [125]

Application of difference and derivative ultra-violet spectrometry for assay of some benzodiazepines. [126]

First derivative spectrophotometric determination of certain drugs in two-component mixtures. [127]

Evaluation of dual-wavelength spectrophotometry for drug level monitoring. [128]

Application of first-derivative spectrophotometry to the determination of certain drugs in single component dosage forms. [129]

Determination of aspirin and salicylic acid in aspirin tablets by second-derivative ultra-violet spectrometry. [130]

Ultra-violet derivative spectrophotometric determination of Cui Xing Ning tablets. [131]

Derivative spectrophotometry and its application in pharmaceutical analysis. [132]

Application of derivative spectrometry in pharmaceutical analysis. II. Determination of guaiphenesin and isoprenaline hydrochloride in aerosol by second-derivative spectrometry and colorimetry. [133]

Determination of phenylpropanolamine hydrochloride in bimin tablets by second-derivative spectrometry. [134]

Study of derivative spectrophotometry for the determination of carbonylhaemoglobin in blood. [135]

Determination of carbonylhaemoglobin in the presence of other blood haemoglobin pigments by visible spectrophotometry. [136]

Determination of certain drugs in multi-component formulations by first-derivative ultra-violet spectrophotometry. [137]

Determination of salicylic acid in aspirin by first-derivative ultra-violet spectrophotometry. [138]

Atropine sulphate analysis by derivative spectroscopy or HPLC. [139]

Determination of some cephalosporins using derivative spectrophotometry. [140]

Determination of coloured substances in soya-bean lecithin (phosphatidylcholine). [141]

First derivative spectrophotometric determination of pyridoxine and meclozine in two-component mixture. [142]

Derivative spectrophotometric determination of praziquantel in tablets. [143]

Studies on derivative spectrophotometry.
I. Theoretical analysis of factors in the resolution of overlapping absorption bands by use of derivative spectrophotometry. [169]

Effect of the degree of polynomials in the Savitzky-Golay method for calculation of second-derivative spectra. [170]

Quantitative analysis by derivative electronic spectroscopy. [171]

Application of derivative spectrophotometry to the study and analysis of complex substances in solution. [172]

Determination of alkylnaphthalenes in petroleum fractions by second-derivative ultra-violet spectrophotometry. [173]

Arson analysis by second-derivative ultra-violet spectrometry. [174]

Derivative spectrophotometry (a literature review). [175]

Ratios of first-derivative maxima and compensated derivative absorption curves. [176]

Experimental

All spectrophotometric measurements presented in this paper were carried out on a new, recently introduced, microprocessor controlled, double-beam UV-Visible scanning spectrophotometer, the Varian DMS 200 [177], equipped with a BMC monitor and a Sekonic S-210 GP thermal printer-plotter. Details of the measurement parameters, chemicals and solvents are given with the corresponding spectral traces. In all cases, unless specifically indicated otherwise, 1-cm pathlength quartz cells were employed.

The DMS 200 spectrophotometer was chosen for this work primarily for its built-in 1st to 6th derivative measurement and display capabilities. It must be noted, however, that many of its other operational and performance characteristics also played a significant, interactive role in the derivative measurements presented here. Some of these interactions and their impact an measured spectral data are discussed in more detail in this section.

Double-beam operation in the DMS 200 is achieved by means of 3-segment 30 Hz rotating choppers, which ratio the sample and reference signals every 33 ms, with a sampling time of only 11 ms between the sequentially ratioed sample and reference signals. The 3-segment design also provides automatic dark current compensation every chopper cycle (i.e. compensation of the statistical background signal produced when no light falls on the photomultiplier detector).

It should be noted that this sequential signal and reference sampling time in single-beam spectrophotometers is usually of the order of tens of seconds, or even longer, and is dependent on the speed of the human operator. Also, most single-beam instruments have no provision, either manual or automatic, for dark current compensation. Furthermore, the indiscriminate, automatic use of microprocessor stored reference baselines (even on the sample cell with solvent) for subsequent sample spectra corrections can lead to unrecognised, incorrect results (quantitatively and qualitatively). This is especially the case for small signal-to-noise ratio (S/N) peaks and shoulders, and particularly in derivative measurements, because single-beam instrument stabilities, even after a 1-hour warm-up period, are generally 6 to 20 times worse than for double-beam instruments.

For example, Figure 23 shows the double-beam DMS 200 stability (at 500 nm, 2 nm spectral band width and 'zero' smoothing time). Figure 24 shows the stability of the DMS 200 operated in the single-beam mode, but with the advantage of automatic dark current compensation.

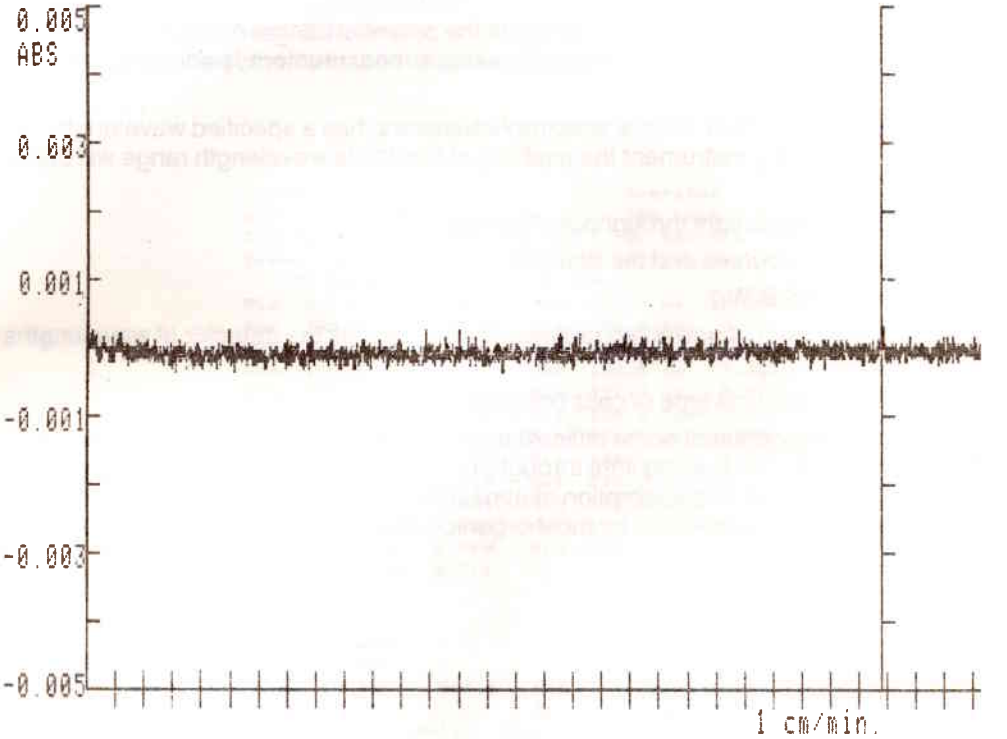

Figure 23

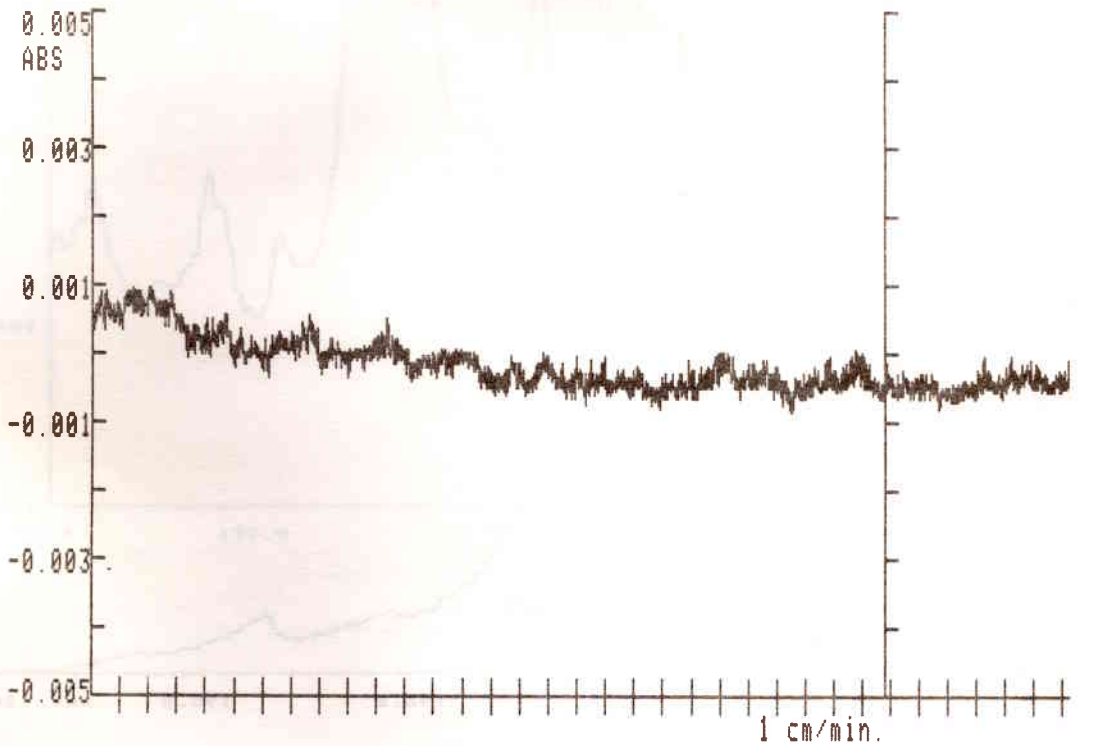

Figure 24

Nevertheless, the latter shows some drift and highlights the potential danger of storing a single-beam instrument baseline and then using it for correction on sample measurements performed minutes or hours later.

The DMS 200, like many modern UV-Visible spectrophotometers, has a specified wavelength range of 190 to 900 nm. However, on any instrument the usability of the whole wavelength range will depend on many factors, such as:

The overall optical design and its light throughput efficiency.

The performance of the light sources and the detector.

The slit spectral bandwidth (S.B.W.).

The stray light level (the amount of unwanted radiation which reaches the detector at wavelengths other than that being measured).

The type of sample, solvent and the type of cells being used.

The region below 220 nm may present some difficulties on all spectrophotometers, because of increasing levels of stray light, decreasing light throughput and decreasing detector response, which is aggravated by the increasingly strong absorption of atmospheric oxygen, particularly below 200 nm (Figure 25), and the very strong absorption by most organic substances, including many useful solvents.

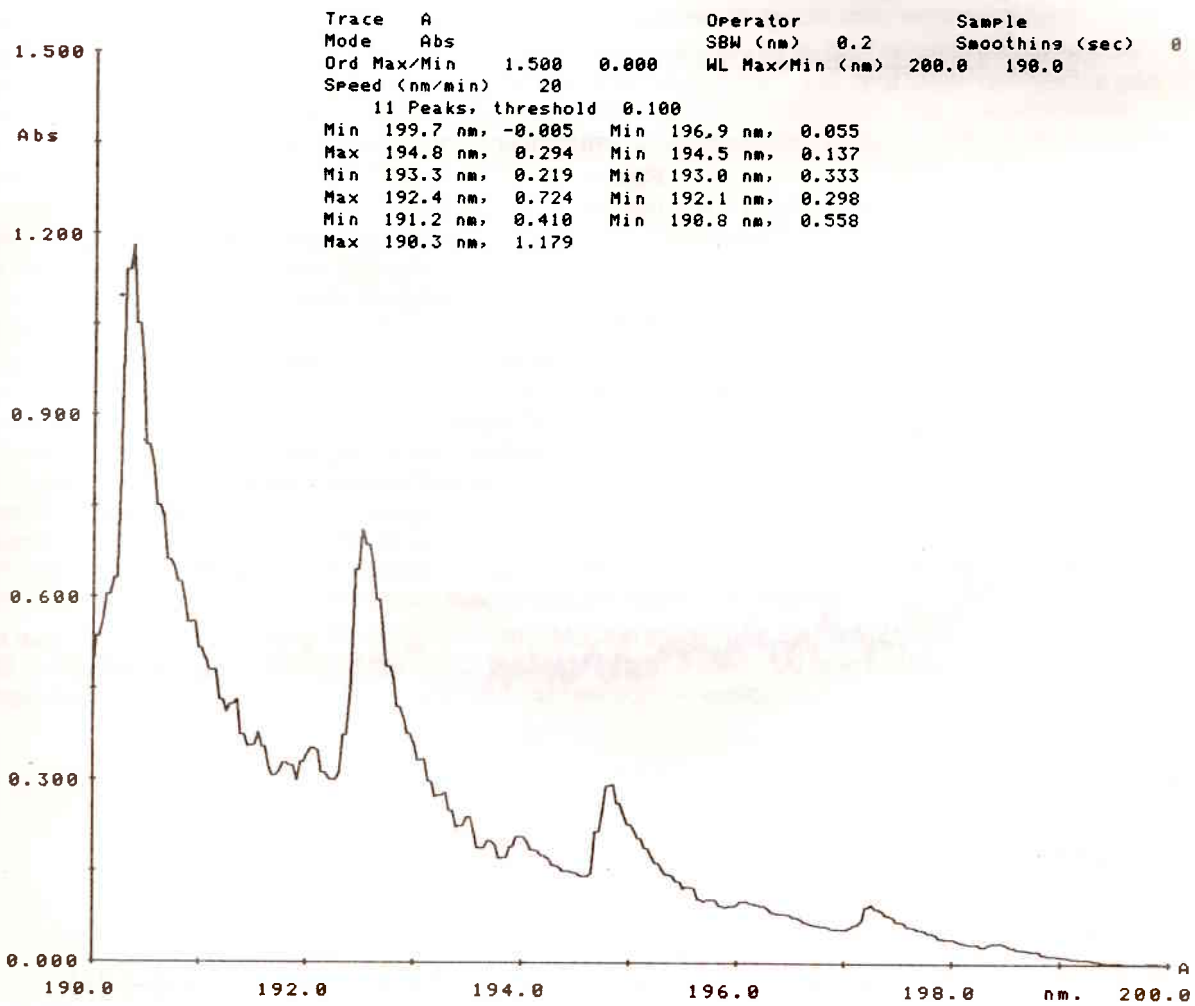

Figure 25

However, the low stray light of the DMS 200 plus the excellent energy throughput, even at narrow S.B.W. settings result in low noise and enable measurements to be made successfully in this 'difficult' region, as illustrated by the spectrum of cyclopentanone vapor, measured with 0.2 nm S.B.W. slits (Figure 26).

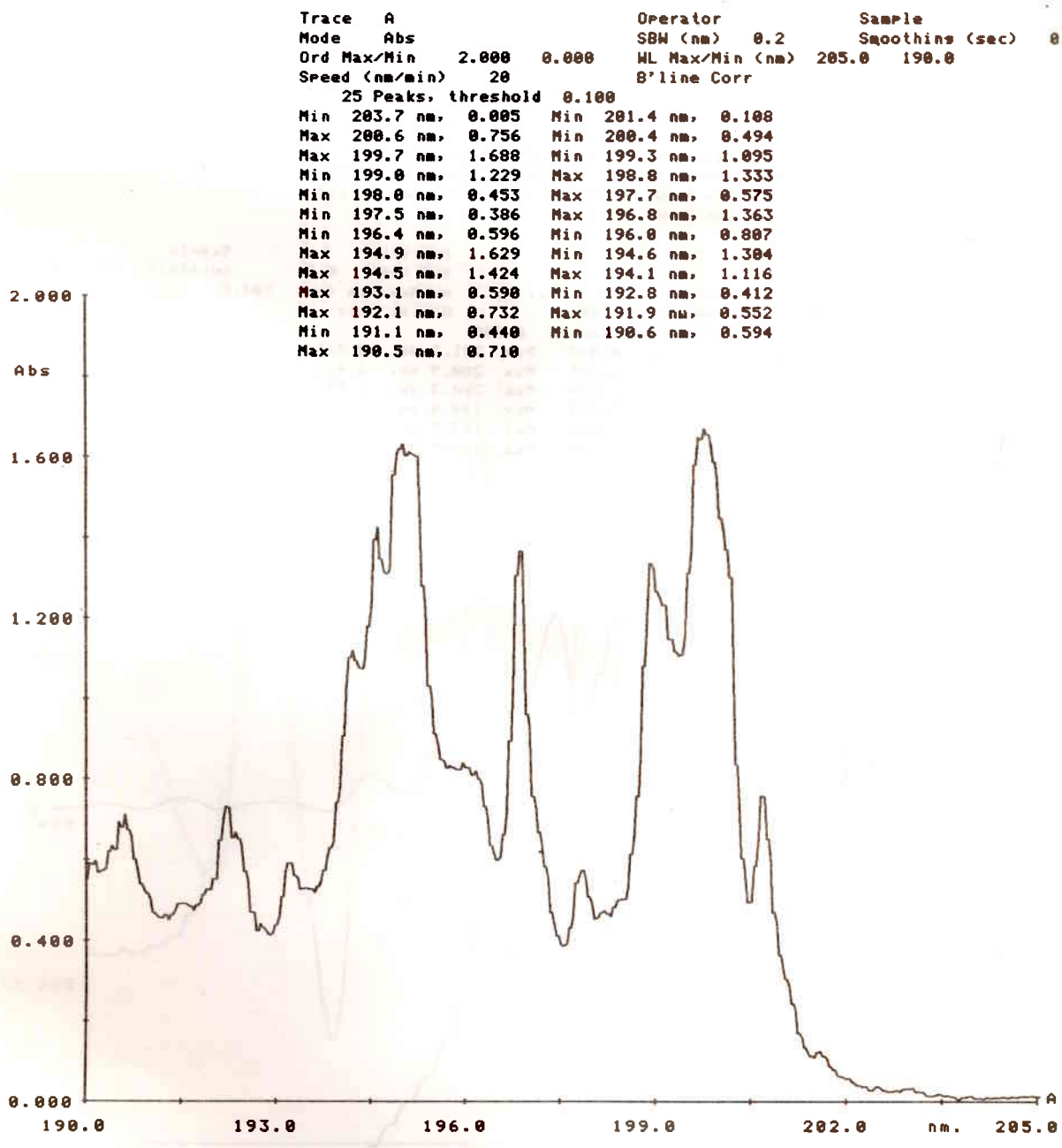

Figure 26

In such a spectrum the peaks and shoulders can be further resolved and more clearly identified by the use of higher derivatives. For example, the progressively improved resolution of the 3 peaks in the region of 198 nm to 203 nm is clearly shown by the 2nd and the 4th derivative traces in Figures 27 and 28 respectively.

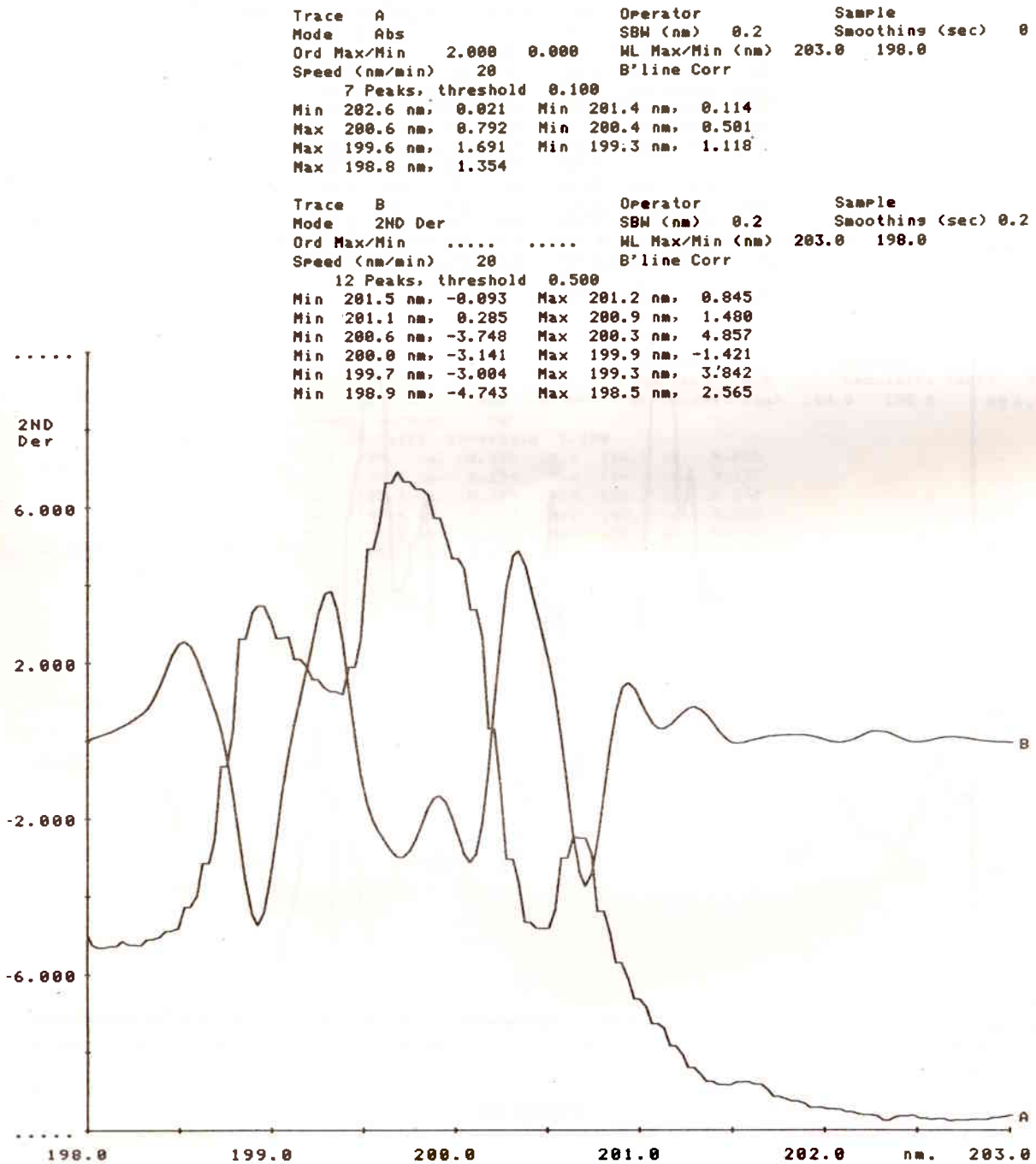

Figure 27

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   0.2        Smoothing (sec)   0
Ord Max/Min     2.000    0.000     WL Max/Min (nm)  203.0    198.0
Speed (nm/min)     20              B'line Corr
      6 Peaks, threshold  0.100
Min  202.6 nm,  0.023   Max  200.6 nm,  0.754
Min  200.4 nm,  0.518   Max  199.6 nm,  1.691
Min  199.3 nm,  1.118   Max  198.9 nm,  1.347
```

```
Trace   B                          Operator              Sample
Mode    4TH Der                    SBW (nm)   0.2        Smoothing (sec) 0.2
Ord Max/Min     .....    .....     WL Max/Min (nm)  203.0    198.0
Speed (nm/min)     20              B'line Corr
     17 Peaks, threshold  0.500
Min  202.7 nm, -0.251   Max  202.4 nm,  0.433
Min  202.2 nm, -0.579   Max  202.0 nm,  0.482
Min  201.8 nm, -0.281   Max  201.5 nm,  1.062
Min  201.2 nm, -1.465   Max  201.1 nm,  1.686
Min  200.9 nm, -6.125   Max  200.7 nm,  .....
Min  200.3 nm,  .....   Max  200.1 nm,  9.186
Min  199.8 nm, -3.118   Max  199.5 nm,  4.638
Min  199.3 nm, -8.886   Max  198.9 nm,  .....
Min  198.6 nm, -4.195
```

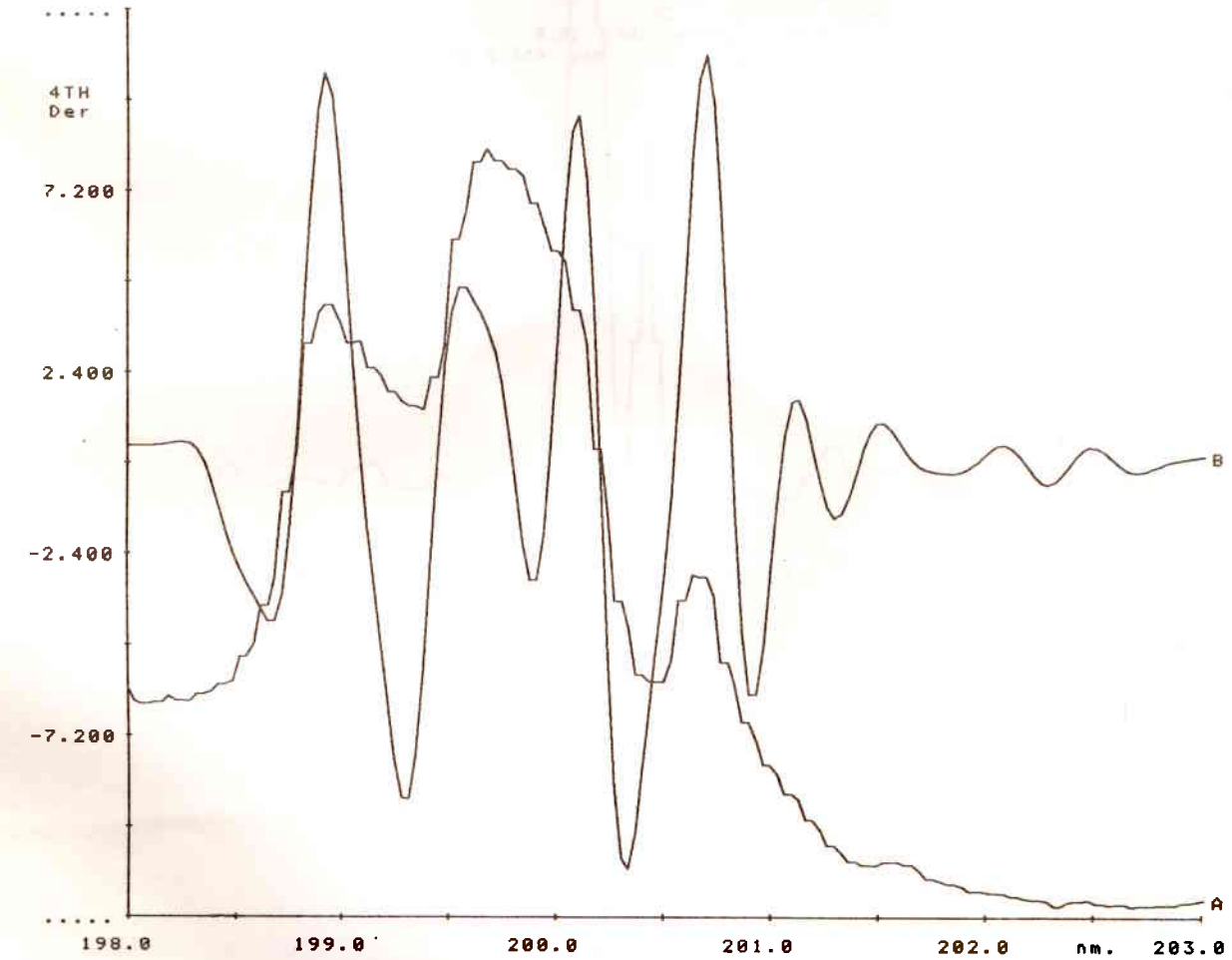

Figure 28

The asymmetrical peaks at 200.6 nm and 199.6 nm are doublet peaks.

Wavelength accuracy and wavelength repeatability also play an important role, particularly in spectral characterization and for spectral comparisons of pure substances and for subsequent archiving. Generally, small deviations in wavelength accuracy are to be expected and are not a serious handicap, since such deviations can be readily measured and corrected for, as long as they are constant over the entire wavelength range of interest.

The 656.10 nm and 486.00 nm emission lines from the deuterium arc UV light source, found in most instruments, are particularly useful for routine, fast checks of wavelength accuracy [178].

On the DMS 200 the wavelength accuracy routine check can be made quickly, using the built-in deuterium arc source in the single beam energy mode, with the automatic source change programmed to occur above 656.1 nm (660 to 700 nm for example). A typical routine wavelength accuracy calibration check is shown in Figures 29 and 30.

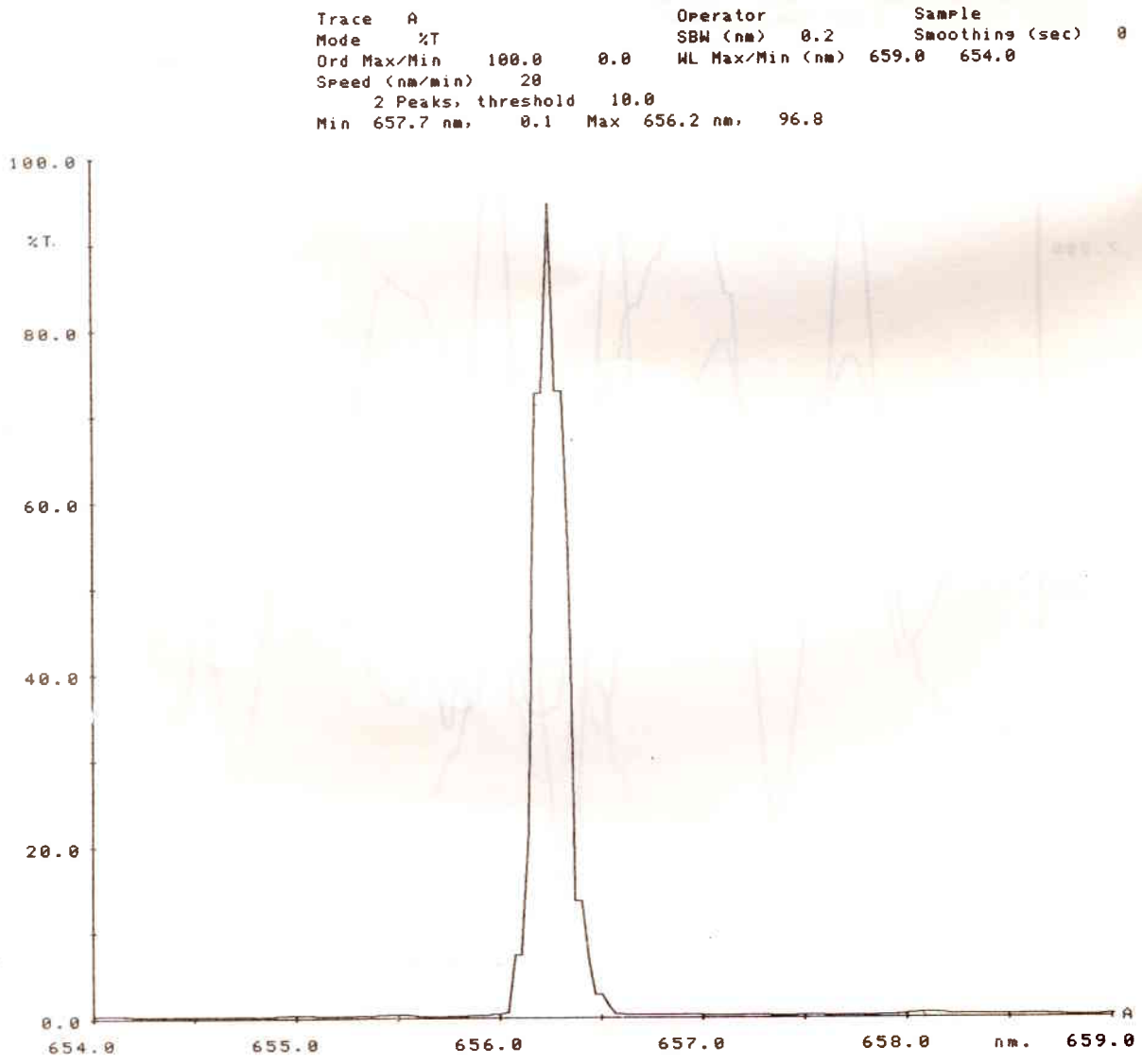

Figure 29

```
Trace   A                          Operator            Sample
Mode     %T                        SBW (nm)   0.2      Smoothing (sec)
Ord Max/Min     100.0      0.0     WL Max/Min (nm)  489.0   484.0
Speed (nm/min)     20
     2 Peaks, threshold    10.0
Min  488.5 nm,     4.5   Max  486.1 nm,    99.6
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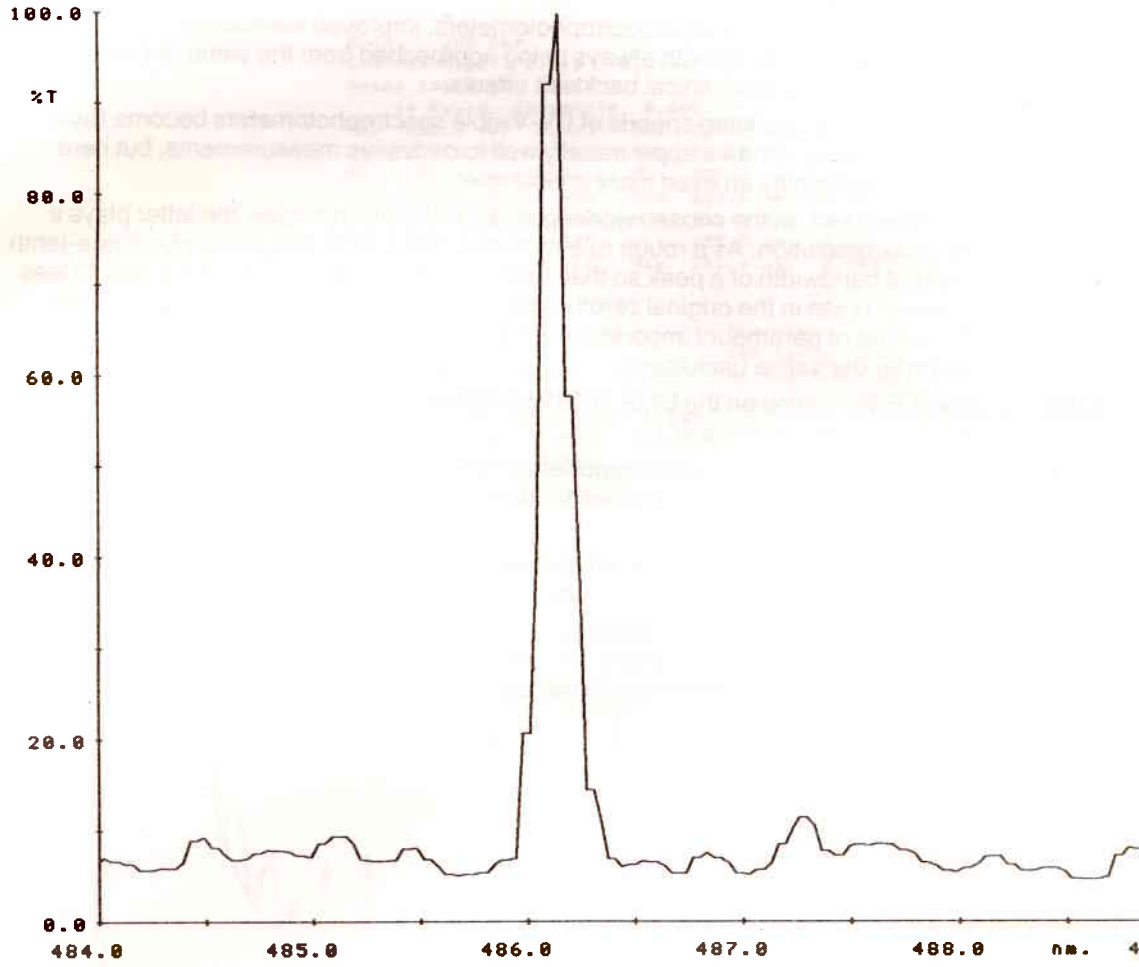

Figure 30

An additional benefit obtained from such a check is an indication of the approximate resolution achieved with the selected S.B.W. That is, the band width at peak-half-height gives a good indication of the spectral slit width.

Wavelength repeatability is probably an even more critical parameter for ensuring reproducible photometric measurements, particularly in quantitative work at fixed wavelengths, ideally at peak maxima, but often on the sides of peaks.

In instruments, such as the DMS 200 and Cary Spectrophotometers, improved wavelength reproducibility is due to the selected wavelength always being approached from the same direction (from longer wavelengths), thus minimizing mechanical backlash effects.

This is particularly important as the scanning speeds of UV-Visible spectrophotometers become faster and faster. Of course, the foregoing remarks apply equally well to derivative measurements, but here performance criteria such as noise play an even more critical role.

Noise will be very much dependent on the chosen working slit S.B.W., and inevitably the latter plays a decisive role in spectral peak resolution. As a rough rule-of-thumb, the S.B.W. should be about one-tenth or less of the 'true' or natural bandwidth of a peak so that the error in the peak absorbance is kept to less than 0.5 % [179]. The level of noise in the original zeroth order absorbance spectrum, which increases with decreasing S.B.W., will be of paramount importance for subsequent derivative calculations — the limiting factor for higher order derivative usefulness.

The completely variable S.B.W. setting on the DMS 200 (from 0.2 nm to 4.0 nm), provides a fairly wide range of control over both resolution and noise.

Further noise control is provided through a built-in proprietary noise filter, which is operator accessible via the selection of one of three digital smoothing times for derivative calculations (0.2, 1, and 5 seconds).

Finally, discrimination against noise peaks is provided by the peak threshold facility which enables the operator to select only those peaks which are of significance for printout.

The interplay of these operation parameters is illustrated by, for example, the absorbance and second derivative spectra of a broad peak (Figure 31), where the 1-second smoothed 2nd derivative trace minimum shows excellent wavelength agreement with the 'zero' smoothed absorbance trace maximum.

```
Trace   A                       Operator            Sample
Mode    Abs                     SBW (nm)   2.0      Smoothing (sec)   0
Ord Max/Min    1.000   0.000    WL Max/Min (nm)  350.0   250.0
Speed (nm/min)   100
     3 Peaks, threshold  0.200
Min  349.6 nm,  0.011   Max  301.0 nm,  0.527
Min  263.1 nm,  0.132

Trace   B                       Operator            Sample
Mode    2ND Der                 SBW (nm)   2.0      Smoothing (sec) 0.2
Ord Max/Min    0.010  -0.010    WL Max/Min (nm)  350.0   250.0
Speed (nm/min)   100
     14 Peaks, threshold  0.005
Max  329.6 nm,  0.003   Max  312.0 nm, -0.001
Min  310.8 nm, -0.007   Max  309.8 nm,  0.002
Min  302.9 nm, -0.007   Min  300.6 nm, -0.006
Min  295.8 nm, -0.004   Min  289.0 nm, -0.003
Min  274.2 nm, -0.002   Min  261.5 nm,  0.004
Min  259.3 nm,  0.005   Min  255.5 nm,  0.016
Min  253.6 nm,  0.023   Max  251.6 nm,  0.049


Trace   C                       Operator            Sample
Mode    2ND Der                 SBW (nm)   2.0      Smoothing (sec)   1
Ord Max/Min    0.010  -0.010    WL Max/Min (nm)  350.0   250.0
Speed (nm/min)   100
     3 Peaks, threshold  0.005
Max  325.5 nm,  0.003   Min  301.1 nm, -0.004
Max  252.9 nm,  0.021
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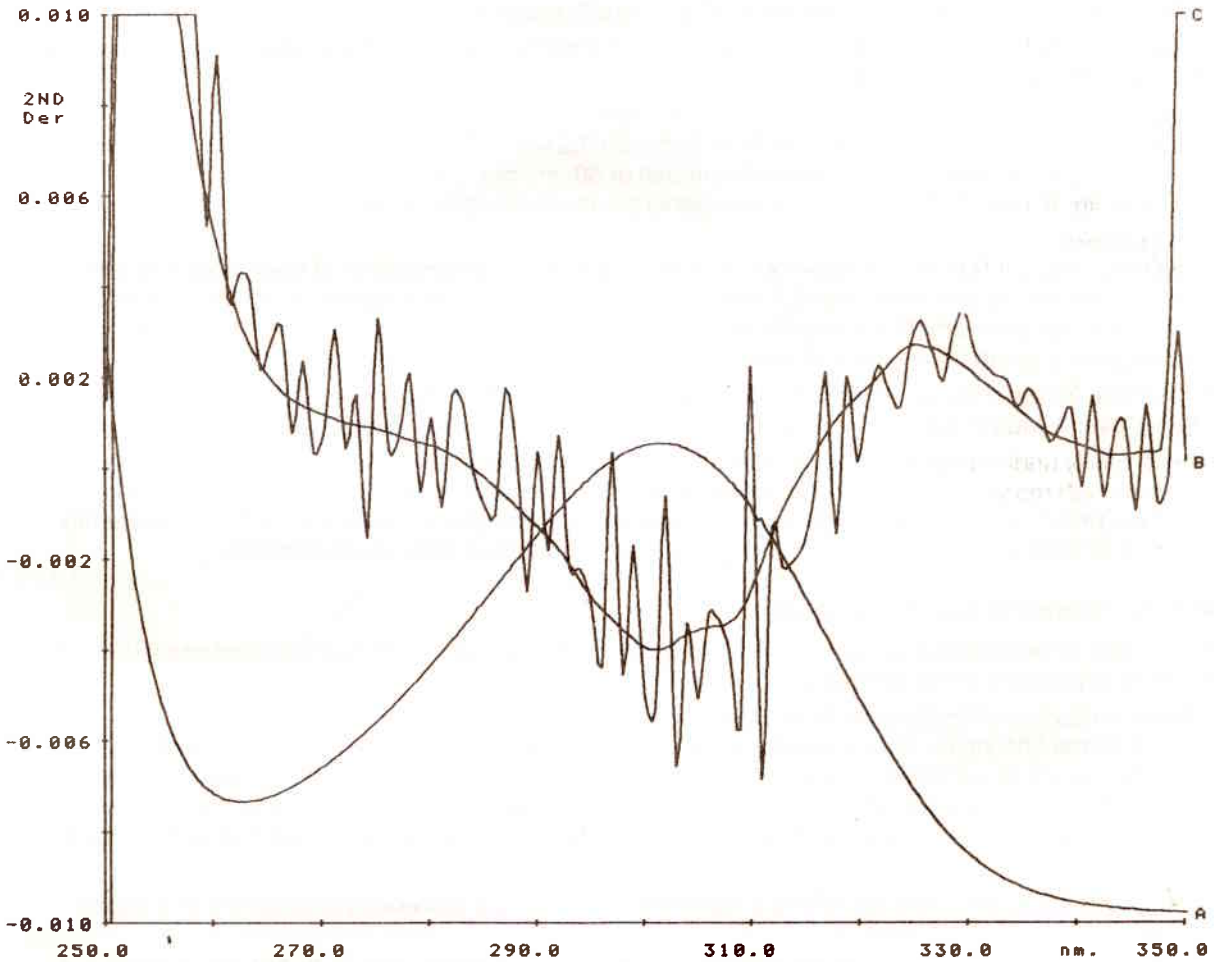

Figure 31

The factors which affect derivative measurements can be summarized as:

The type of sample, i.e. having sharp or broad spectral features, requiring narrow or wide S.B.W.'s, respectively.

The sample concentration, which will determine the absorbance level, and therefore produce high or low noise situations.

The wavelength region in which the sample absorbs, again producing either high or low noise conditions.

The selectable instrument (DMS 200) operating parameters which will determine the quality of derivative data are:

The scanning speed, which will determine the data sampling interval ($\delta\lambda$) for derivative calculations:

Scan Speed (nm/min)	$\delta\lambda$Interval (nm)
20	0.2
50	0.5
100	1.0
200	2.0
500	5.0
1000	10.0

The slit S.B.W., which will determine the resolution and the level of noise:

from 0.2 nm to 4.0 nm, selectable in 0.1 nm steps.

The smoothing filter times, which will determine the number of collected data points taken into the calculation of each derivative point:

Selectable digital smoothing filter times (0.2, 1, and 5 seconds).

Thus, as a rough guide, the following 'trading rules' for the optimization of derivative measurements on the DMS 200 can be put forward:

Narrow Peaks:
For optimum resolution a narrow slit S.B.W. between 0.2 nm — 0.5 nm, should be used, together with a relatively slow scanning speed, ideally 20 nm/min or 50 nm/min, and definitely not faster than 100 nm/min. A 1-second smoothing filter is generally the most appropriate.

Broad Peaks:
Relatively wide slit S.B.W.'s, 1 nm — 4 nm, should be used for minimization of noise, together with medium smoothing filter time (1 or 0.2 second), and medium scanning speeds of 100 nm/min or 200 nm/min for peak amplitude amplification. Unless a broad peak is a composite of several overlapping narrow peaks, going above the 2nd derivative may often prove to be highly questionable.

In general, for rapid survey scans, that is, fast scan speeds of 500 nm/min or 1000 nm/min (large $\delta\lambda$ steps), over a wide wavelength range, derivative measurements are a rather futile exercise.

These easily understandable and controllable optimization parameters, the visual display of spectra on a medium-high resolution CRT screen, together with the extensive on-screen spectral manipulation facilities, prior to print-out on a high-resolution graphics printer-plotter, make the DMS 200 eminently suitable for both normal and derivative UV-Visible spectrophotometric measurements.

Measurements and Discussion

In this section are presented various examples of derivative measurements which illustrate a few of the areas of application of the technique.

Characterization of Individual Pure Compounds

Very often the UV-Visible spectrophotometric technique, on its own, has not been very useful for the characterization of substances, even when pure, and particularly in solutions. The relative non-specifity has hindered its wide application to qualitative analyses. However, the advent of and improvements in derivatization methods have brought new possibilities for the universally used and frequently abused UV-Visible technique.

Today, derivative spectroscopy allows a fresh look to be taken at previously unresolved or partially resolved UV-Visible problems.

Obviously, the field of application for derivative techniques is extremely wide, thus only a few selected examples are presented here.

The study of steroids, using 1st derivative spectroscopy, has been reported by Olson and Alway [9 — Part I], who were, for example, able to identify 6 peaks in the spectrum of testosterone using the zero point crossings. Using the 2nd derivative mode (scan speed of 100 nm/minute and 1 nm S.B.W), the broad, rather featureless zero order spectrum of testosterone (Fluka, purum grade) dissolved in dioxane (Fluka, spectroscopic grade) shows 6 quite distinctive negative peaks in the 280-380 nm region (Figure 32).

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   1.0        Smoothing (sec)   0
Ord Max/Min     1.000   0.000      WL Max/Min (nm)  380.0   280.0
Speed (nm/min)   100               B'line Corr
     24 Peaks, threshold  0.010
Min  379.2 nm,  0.011    Min  373.4 nm,  0.029
Min  368.6 nm,  0.066    Min  350.6 nm,  0.268
Min  343.9 nm,  0.365    Min  340.6 nm,  0.391
Min  336.8 nm,  0.437    Min  332.3 nm,  0.489
Max  329.7 nm,  0.501    Max  317.6 nm,  0.486
Max  315.0 nm,  0.464    Max  313.8 nm,  0.453
Min  310.7 nm,  0.418    Max  310.5 nm,  0.430
Max  309.9 nm,  0.417    Max  308.4 nm,  0.402
Max  304.9 nm,  0.368    Max  302.9 nm,  0.347
Max  296.7 nm,  0.297    Max  295.2 nm,  0.288
Max  293.0 nm,  0.274    Max  291.2 nm,  0.263
Max  288.1 nm,  0.249    Min  283.5 nm,  0.237

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   1.0        Smoothing (sec)   1
Ord Max/Min     0.005  -0.005      WL Max/Min (nm)  380.0   280.0
Speed (nm/min)   100               B'line Corr
     3 Peaks, threshold  0.005
Max  353.5 nm,  0.003    Max  337.3 nm,  0.002
Min  330.2 nm, -0.004
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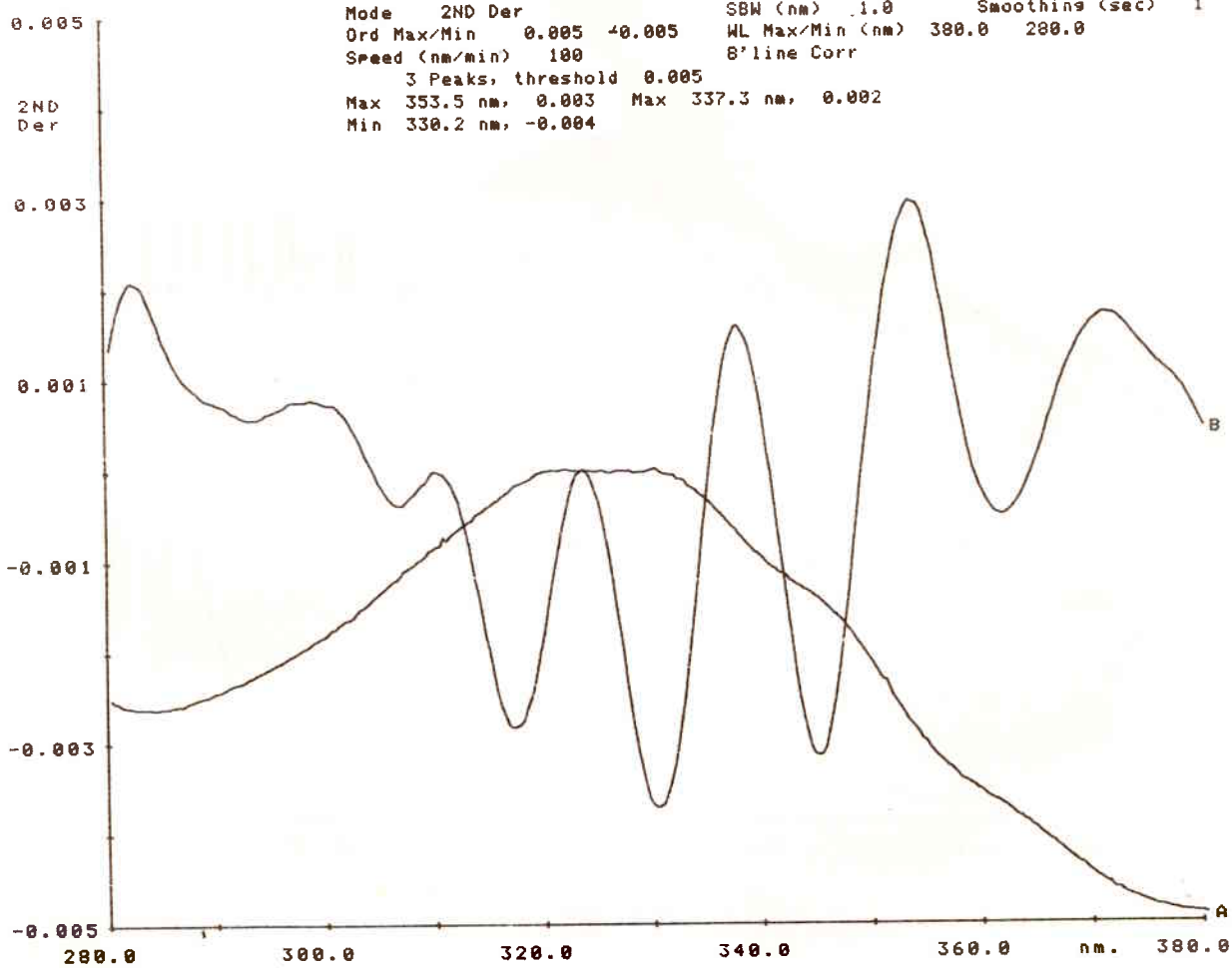

Figure 32

The DMS 200 zoom facility was used to locate the wavelengths of the 5 less intense peaks. The agreement between the two sets of peak wavelengths is acceptable, as the solvents used may have been different:

Negative Peak (2nd Derivative)	Zero Order Crossing (1st Derivative — Ref. 9)
361.8 nm	361 nm
344.7 nm	344 nm
330.2 nm	332 nm
317.2 nm	324 nm
305.6 nm	310 nm
ca. 293.0 nm	287 nm

and indicates the possibilities of derivative spectroscopic characterization of steroids with modern UV-Visible instruments.

An interesting problem in the field of inorganic chemistry is the determination of the exact number and location of peaks in the UV-Visible spectrum of the uranyl ion (UO_2^{++}) in the 330 to 500 nm region. Using derivative techniques, the 19 bands can be very easily resolved and their positions established with some accuracy (Figure 33). Again the zoom facility can be used for the exact location of weaker intensity peaks.

Trace A Operator S.S. Sample $UO_2(NO_3)_2$ in water
Mode Abs SBW (nm) 0.5 Smoothing (sec) 0
Ord Max/Min 2.000 0.000 WL Max/Min (nm) 505.0 325.0
Speed (nm/min) 50 B'line Corr
12 Peaks, threshold 0.050

Min	501.4 nm,	0.001	Min	458.2 nm,	0.051
Min	446.3 nm,	0.110	Min	439.7 nm,	0.167
Min	431.4 nm,	0.250	Min	421.9 nm,	0.321
Max	413.8 nm,	0.412	Max	402.6 nm,	0.362
Max	396.5 nm,	0.277	Max	385.5 nm,	0.181
Min	364.6 nm,	0.121	Min	348.9 nm,	0.181

Trace B Operator Sample
Mode 2ND Der SBW (nm) 0.5 Smoothing (sec) 1
Ord Max/Min 0.015 -0.015 WL Max/Min (nm) 505.0 325.0
Speed (nm/min) 50 B'line Corr
19 Peaks, threshold 0.005

Max	433.2 nm,	0.003	Min	425.9 nm,	-0.005
Max	419.4 nm,	0.005	Min	412.9 nm,	-0.007
Max	406.8 nm,	0.005	Min	401.0 nm,	-0.006
Max	395.0 nm,	0.004	Min	389.6 nm,	-0.003
Max	373.9 nm,	0.003	Min	368.8 nm,	-0.003
Max	363.5 nm,	0.004	Min	358.5 nm,	-0.005
Max	353.5 nm,	0.005	Min	349.9 nm,	-0.001
Max	345.1 nm,	0.008	Min	341.1 nm,	-0.003
Max	337.0 nm,	0.018	Min	332.1 nm,	-0.014
Max	328.0 nm,	0.024			

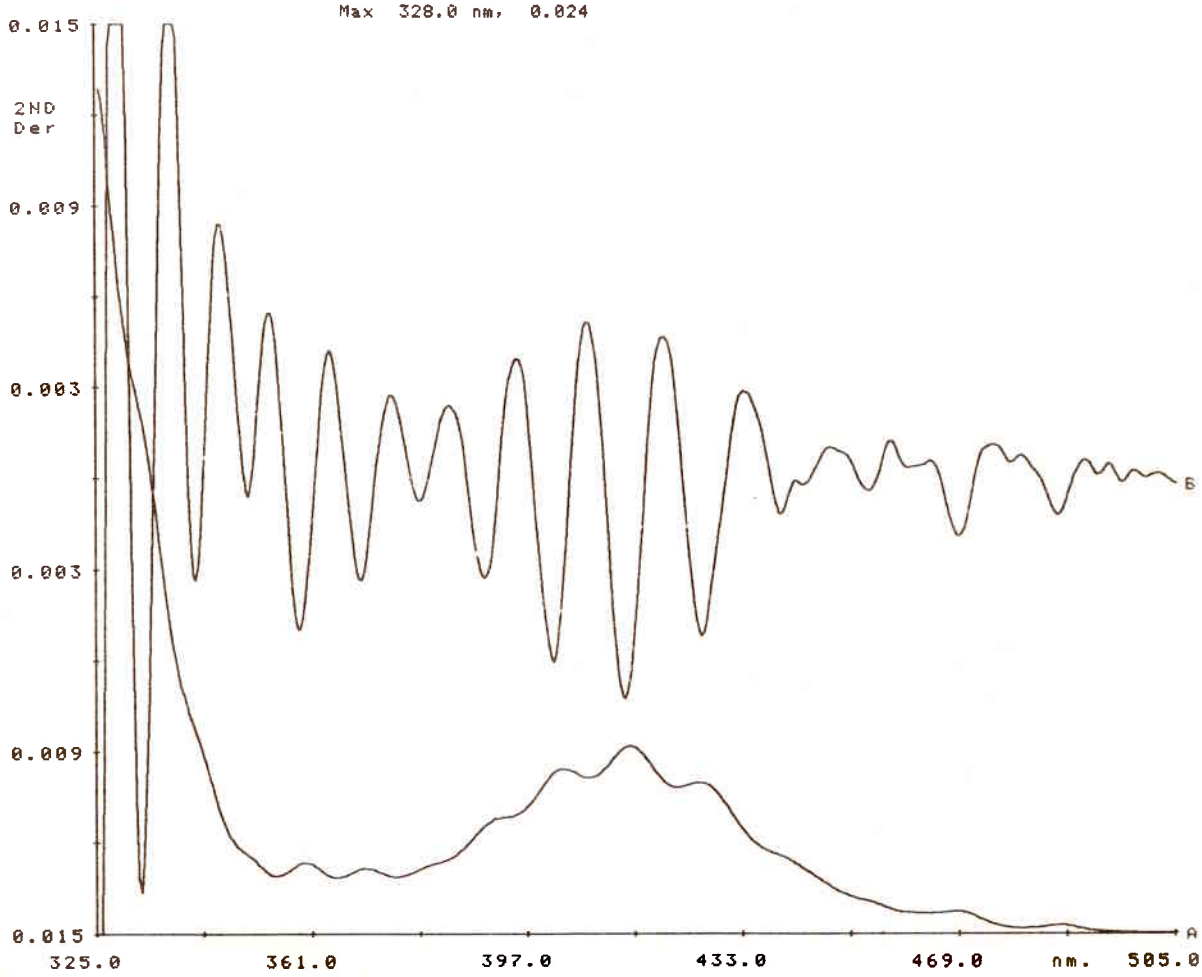

Figure 33

Trace A Operator Sample
Mode Abs SBW (nm) 0.2 Smoothing (sec) 0
Ord Max/Min 3.000 0.000 WL Max/Min (nm) 400.0 210.0
Speed (nm/min) 50 B'line Corr
21 Peaks, threshold 0.200

Min	379.8 nm,	-0.023	Max	327.4 nm,	0.631
Min	325.0 nm,	0.234	Max	321.6 nm,	0.604
Min	319.5 nm,	0.323	Max	315.5 nm,	0.529
Max	306.8 nm,	0.321	Min	284.4 nm,	0.083
Min	266.1 nm,	2.017	Min	264.2 nm,	2.200
Min	263.3 nm,	2.409	Min	260.6 nm,	2.576
Max	260.4 nm,	2.781	Max	257.0 nm,	2.650
Max	252.8 nm,	2.481	Max	251.0 nm,	2.270
Max	248.9 nm,	2.060	Max	246.8 nm,	1.718
Max	244.2 nm,	1.270	Max	239.3 nm,	0.680
Max	236.4 nm,	0.473			

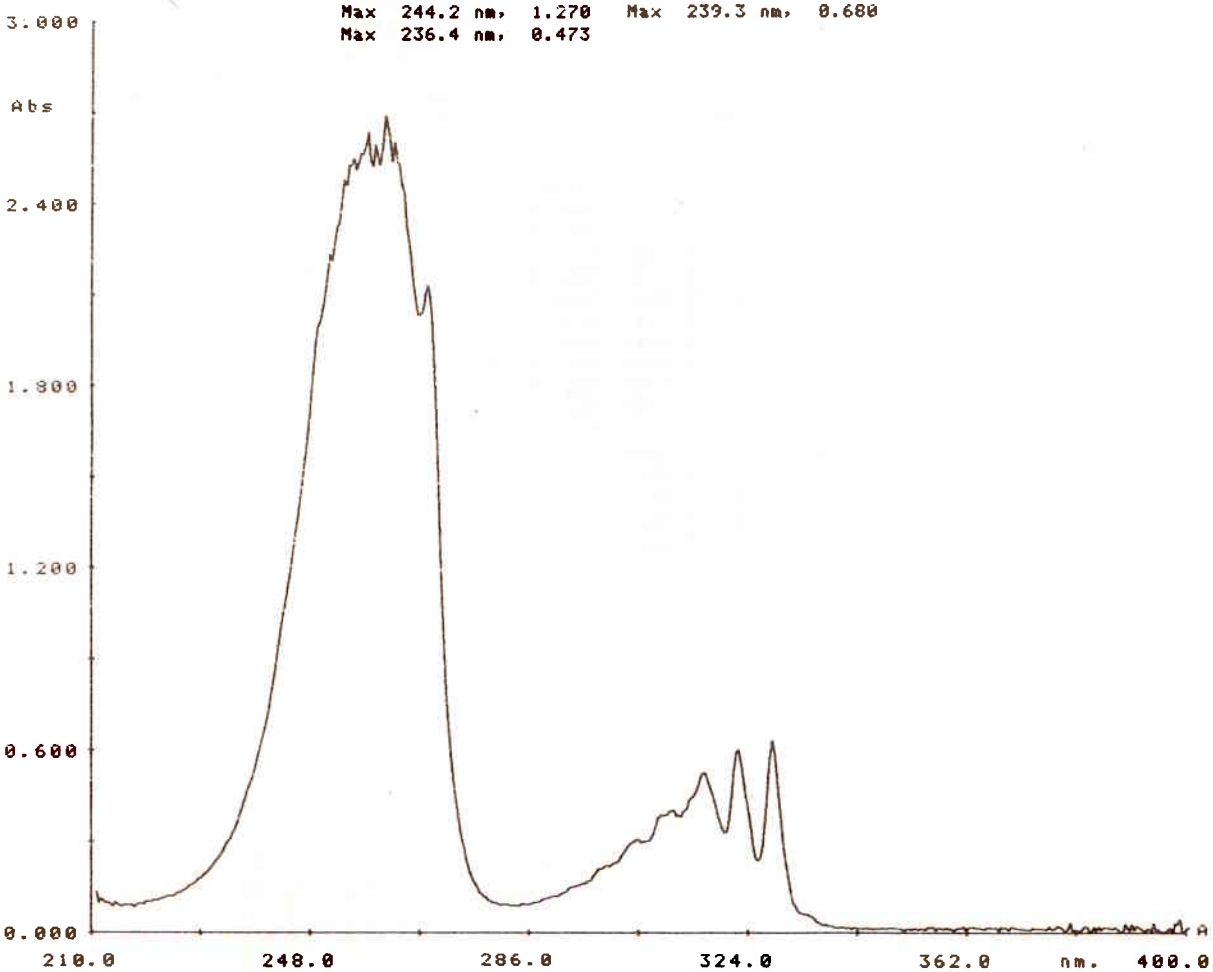

Figure 34

The long wavelength peaks are at 327.5 nm, 321.3 nm and 315.5 nm. However, the 2nd derivative spectrum, at a scanning speed of 20 nm/min, indicates the presence of additional peaks (Figure 35).

```
Trace   A                       Operator            Sample
Mode    Abs                     SBW (nm)   0.2      Smoothing (sec)   0
Ord Max/Min    1.000   0.000    WL Max/Min (nm)  340.0   280.0
Speed (nm/min)    20            B'line Corr
     8 Peaks, threshold  0.200
Min  339.0 nm,  0.010   Min  328.9 nm,  0.377
Max  327.5 nm,  0.627   Min  324.8 nm,  0.233
Max  321.3 nm,  0.601   Min  319.2 nm,  0.324
Max  315.5 nm,  0.529   Max  306.7 nm,  0.321

Trace   B                       Operator            Sample
Mode    2ND Der                 SBW (nm)   0.2      Smoothing (sec)   1
Ord Max/Min    0.150  -0.150    WL Max/Min (nm), 340.0   280.0
Speed (nm/min)    20            B'line Corr
    31 Peaks, threshold  0.005
Max  335.3 nm,  0.004   Min  334.3 nm, -0.003
Min  331.3 nm,  0.012   Max  329.9 nm,  0.027
Min  327.0 nm, -0.140   Max  325.1 nm,  0.074
Min  320.9 nm, -0.093   Max  319.3 nm,  0.073
Max  316.4 nm, -0.007   Min  315.2 nm, -0.038
Max  314.0 nm,  0.014   Min  312.6 nm, -0.005
Max  311.3 nm,  0.014   Min  309.6 nm, -0.010
Max  308.7 nm,  0.001   Min  307.5 nm, -0.017
Max  305.6 nm,  0.014   Min  302.3 nm, -0.008
Max  300.5 nm,  0.010   Max  299.3 nm,  0.003
Min  297.2 nm, -0.004   Max  295.2 nm,  0.004
Min  292.2 nm, -0.003   Max  290.1 nm,  0.003
Min  287.5 nm, -0.004   Max  286.6 nm,  0.004
Min  285.8 nm, -0.002   Max  284.1 nm,  0.003
Min  283.3 nm, -0.003   Max  282.0 nm,  0.004
Min  281.1 nm, -0.003
```

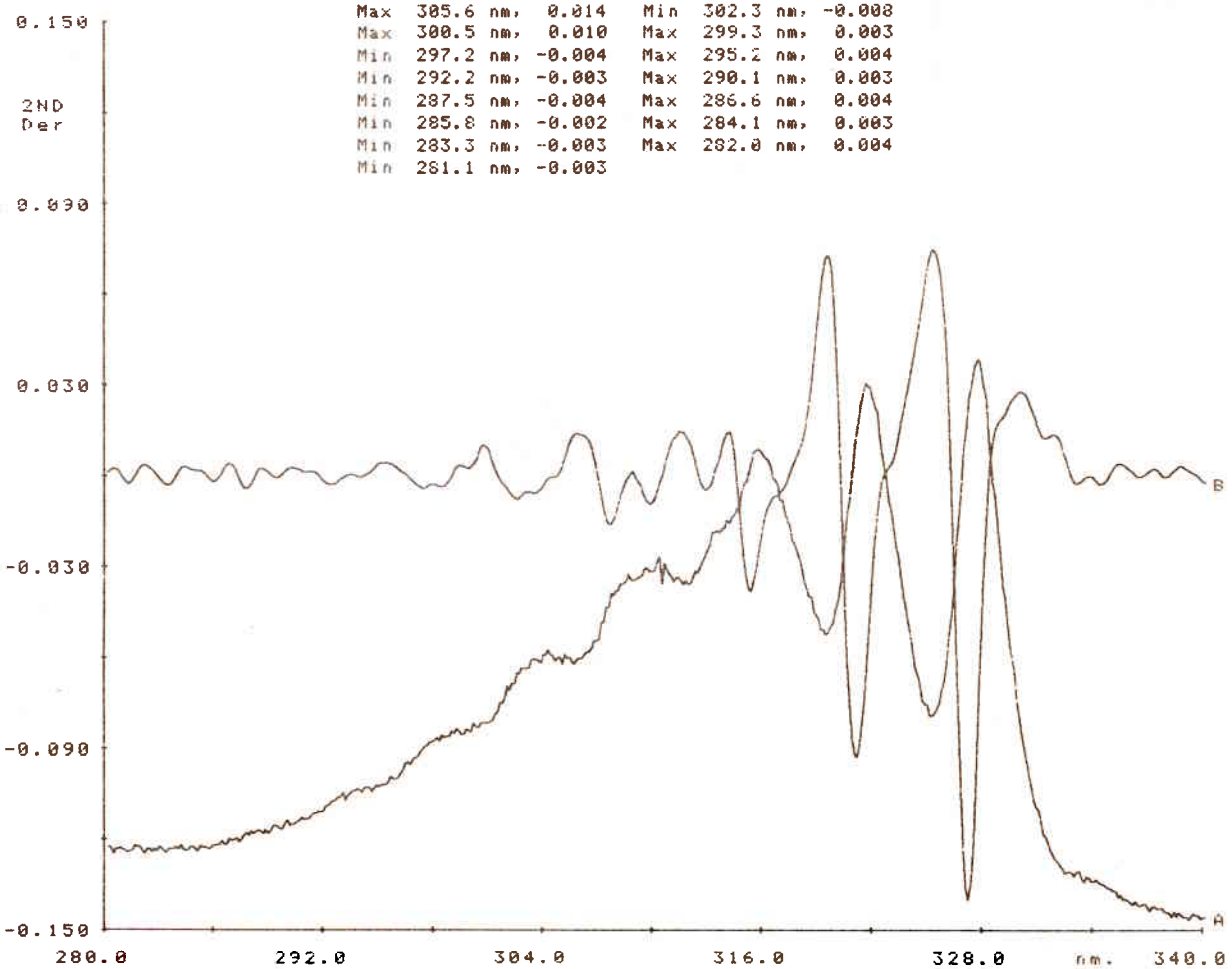

Figure 35

Similarly, the so-called 258 nm peak can be resolved by 2nd derivative spectroscopy into at least 6 quite distinct individual peaks (Figure 36), located at 267.3 nm, 260.3 nm, 256.2 nm, 253.8 nm, 250.2 nm and 247.5 nm.

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   0.2        Smoothing (sec)   0
Ord Max/Min    2.000    0.000      WL Max/Min (nm)  280.0   210.0
Speed (nm/min)    20
    13 Peaks, threshold  0.200
Min  279.7 nm,  0.036   Min  271.9 nm,  0.244
Min  268.2 nm,  0.984   Min  263.8 nm,  1.188
Min  262.4 nm,  1.448   Max  260.5 nm,  1.718
Max  255.1 nm,  1.561   Max  252.9 nm,  1.355
Max  250.7 nm,  1.157   Max  247.7 nm,  0.947
Max  246.0 nm,  0.745   Max  242.8 nm,  0.529
Max  239.1 nm,  0.319

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   0.2        Smoothing (sec)   1
Ord Max/Min    0.100   -0.100      WL Max/Min (nm)  280.0   210.0
Speed (nm/min)    20
    30 Peaks, threshold  0.005
Min  279.1 nm, -0.000   Min  272.8 nm,  0.007
Min  271.3 nm,  0.015   Max  270.1 nm,  0.042
Min  267.3 nm, -0.079   Max  265.5 nm,  0.041
Max  263.9 nm,  0.029   Min  260.3 nm, -0.088
Max  258.8 nm,  0.034   Max  257.7 nm,  0.028
Min  256.2 nm, -0.012   Max  255.2 nm,  0.010
Min  253.8 nm, -0.056   Max  251.4 nm,  0.029
Min  250.2 nm, -0.010   Max  249.1 nm,  0.007
Min  247.5 nm, -0.022   Max  245.9 nm,  0.012
Min  243.4 nm, -0.000   Max  242.7 nm,  0.005
Min  241.9 nm, -0.005   Min  240.7 nm, -0.001
Max  239.8 nm,  0.007   Min  235.6 nm, -0.002
Max  234.3 nm,  0.003   Max  221.5 nm,  0.002
Min  216.4 nm, -0.003   Max  215.6 nm,  0.005
Min  214.6 nm, -0.003   Max  211.6 nm,  0.006
```

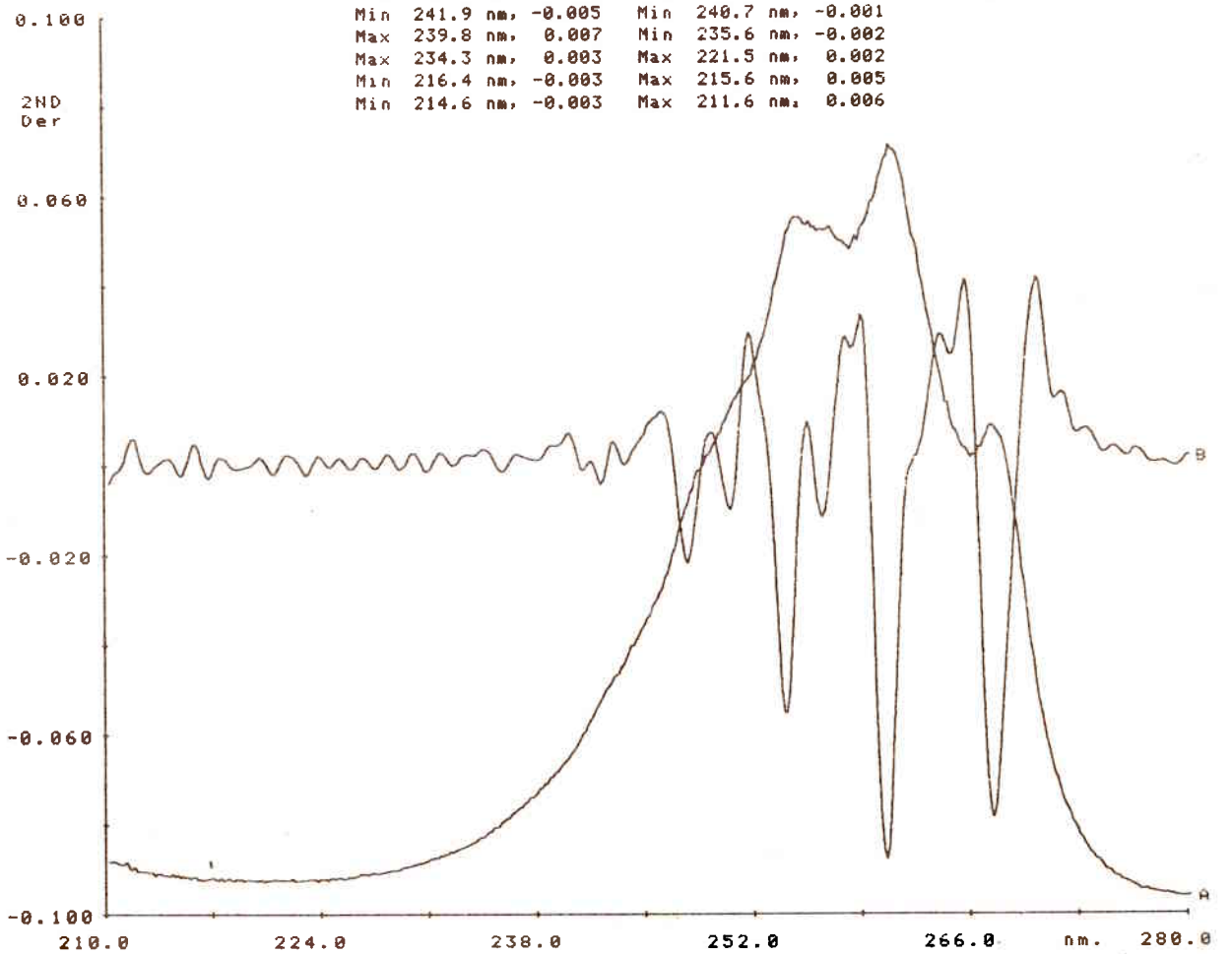

Figure 36

In the spectrum of the isomeric diazine, pyrimidine, the two peak groups are shifted towards shorter wavelengths and show less resolved fine structure in the zero order UV spectrum (Figure 37).

Trace	A		Operator		Sample
Mode	Abs		SBW (nm)	0.2	Smoothing (sec) 0
Ord Max/Min	3.000	0.000	WL Max/Min (nm)	400.0	210.0
Speed (nm/min)	50		B'line Corr		

32 Peaks, threshold 0.200

Min	350.7 nm,	-0.004	Min	316.4 nm,	0.322
Min	308.4 nm,	0.683	Max	298.1 nm,	0.980
Max	280.0 nm,	0.767	Min	262.9 nm,	0.407
Min	251.1 nm,	2.453	Min	250.1 nm,	2.536
Min	248.6 nm,	2.588	Max	248.1 nm,	2.838
Min	247.3 nm,	2.603	Max	245.8 nm,	2.855
Min	245.4 nm,	2.618	Max	244.8 nm,	2.850
Min	243.9 nm,	2.641	Max	242.6 nm,	2.878
Min	242.3 nm,	2.638	Max	241.7 nm,	2.918
Min	241.2 nm,	2.666	Max	241.1 nm,	2.978
Max	239.7 nm,	2.874	Min	235.9 nm,	2.667
Max	235.4 nm,	2.897	Max	233.8 nm,	2.799
Max	231.4 nm,	2.513	Max	229.7 nm,	2.348
Max	228.3 nm,	2.130	Max	227.4 nm,	1.923
Max	225.8 nm,	1.641	Min	221.9 nm,	1.426
Min	217.9 nm,	1.602	Max	212.4 nm,	1.951

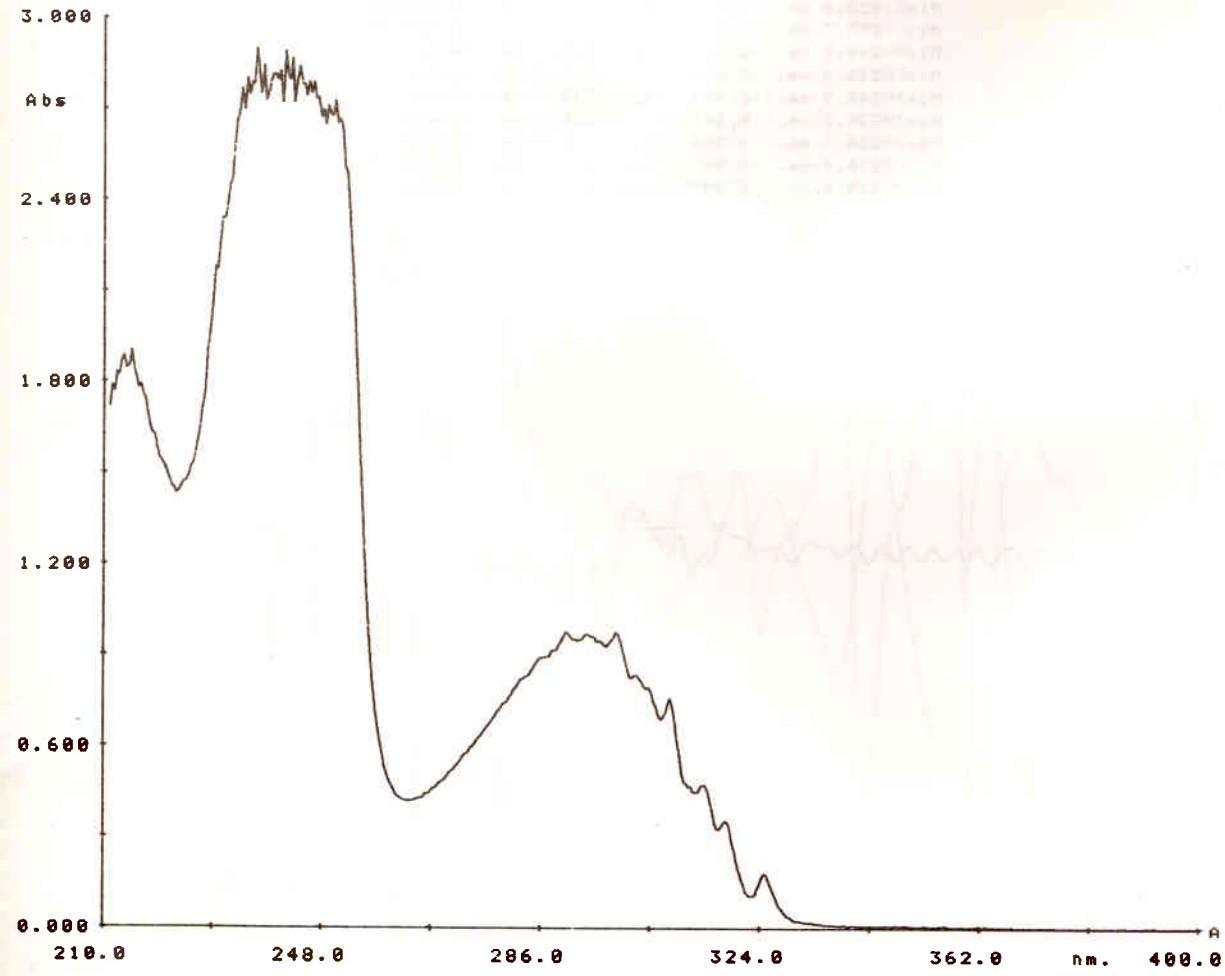

Figure 37

Again, however, it is possible to resolve these peaks into their component peaks with the derivative technique. The effect of scanning speed on the resolution is quite dramatically illustrated in Figure 38 and Figure 39, the latter, at 20 nm/min scanning speed, showing resolved fine structure.

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   0.2        Smoothing (sec)   0
Ord Max/Min    1.500    0.000      WL Max/Min (nm)  340.0    260.0
Speed (nm/min)    50              B'line Corr
      5 Peaks, threshold  0.200
Min  339.1 nm,  0.005   Min  316.2 nm,  0.322
Min  305.9 nm,  0.686   Max  298.1 nm,  0.980
Max  279.9 nm,  0.766

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   0.2        Smoothing (sec)   1
Ord Max/Min    0.060   -0.060      WL Max/Min (nm)  340.0    260.0
Speed (nm/min)    50              B'line Corr
    20 Peaks, threshold  0.005
Min  337.5 nm, -0.001   Max  326.2 nm,  0.012
Min  323.5 nm, -0.028   Max  320.1 nm,  0.032
Min  316.8 nm, -0.029   Max  314.8 nm,  0.020
Min  312.7 nm, -0.026   Max  309.6 nm,  0.037
Min  306.8 nm, -0.045   Max  304.7 nm,  0.019
Min  302.4 nm, -0.011   Max  299.8 nm,  0.019
Min  297.4 nm, -0.031   Max  295.2 nm,  0.011
Min  292.4 nm, -0.007   Max  290.5 nm,  0.002
Min  288.4 nm, -0.011   Max  286.1 nm,  0.005
Min  283.9 nm, -0.004   Min  274.5 nm, -0.001
```

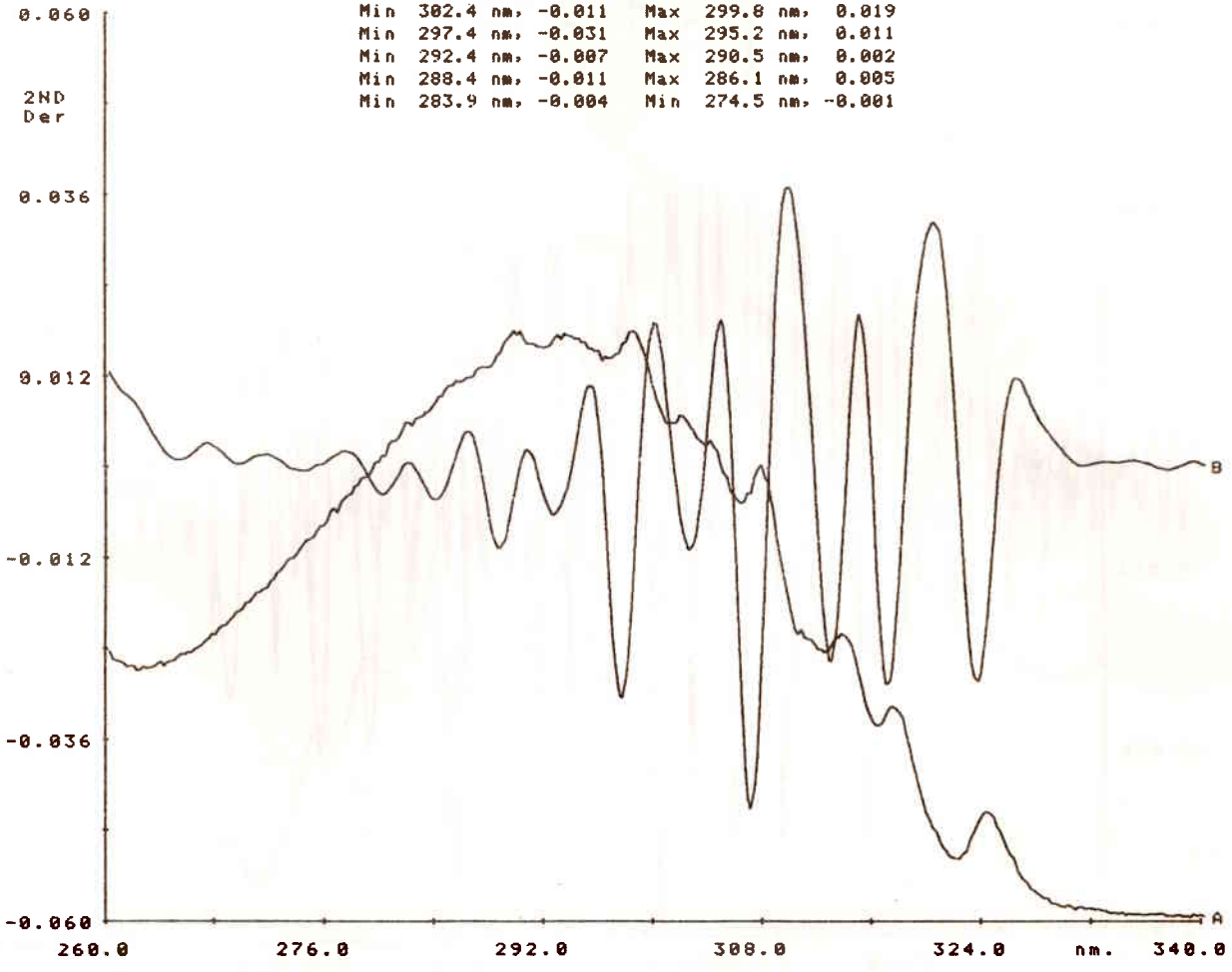

Figure 38

Trace	A	Operator	Sample
Mode	Abs	SBW (nm) 0.2	Smoothing (sec) 0
Ord Max/Min	1.500 0.000	WL Max/Min (nm)	340.0 260.0
Speed (nm/min)	20	B'line Corr	

33 Peaks, threshold 0.050

Min	338.3 nm,	0.004	Min	327.3 nm,	0.057
Min	325.7 nm,	0.116	Max	324.1 nm,	0.179
Min	322.0 nm,	0.100	Min	320.2 nm,	0.156
Min	319.1 nm,	0.227	Min	316.4 nm,	0.318
Min	315.0 nm,	0.378	Min	314.5 nm,	0.429
Min	310.3 nm,	0.466	Min	309.1 nm,	0.582
Min	308.3 nm,	0.696	Max	307.4 nm,	0.751
Min	305.9 nm,	0.686	Min	304.8 nm,	0.739
Min	303.3 nm,	0.780	Min	300.6 nm,	0.812
Min	300.0 nm,	0.860	Min	299.3 nm,	0.916
Max	298.4 nm,	0.979	Min	296.8 nm,	0.918
Max	289.6 nm,	0.981	Max	288.3 nm,	0.940
Max	285.6 nm,	0.899	Max	283.7 nm,	0.850
Max	280.9 nm,	0.801	Max	279.3 nm,	0.745
Max	276.8 nm,	0.695	Max	275.2 nm,	0.645
Max	273.1 nm,	0.598	Max	271.3 nm,	0.548
Max	268.8 nm,	0.498			

Trace	B	Operator	Sample
Mode	2ND Der	SBW (nm) 0.2	Smoothing (sec)
Ord Max/Min	0.060 -0.060	WL Max/Min (nm)	340.0 260.0
Speed (nm/min)	20	B'line Corr	

39 Peaks, threshold 0.005

Min	339.3 nm,	-0.002	Max	325.8 nm,	0.008
Min	323.7 nm,	-0.028	Max	321.9 nm,	0.021
Max	320.2 nm,	0.013	Min	317.2 nm,	-0.036
Max	315.5 nm,	0.035	Min	313.6 nm,	-0.035
Max	311.8 nm,	0.016	Min	310.9 nm,	-0.001
Max	309.4 nm,	0.036	Min	307.3 nm,	-0.052
Max	305.7 nm,	0.033	Min	303.5 nm,	-0.011
Max	302.6 nm,	0.006	Min	301.6 nm,	-0.013
Max	300.0 nm,	0.032	Min	298.2 nm,	-0.033
Max	296.2 nm,	0.019	Min	295.0 nm,	-0.004
Max	293.6 nm,	0.003	Min	292.7 nm,	-0.014
Max	291.0 nm,	0.016	Min	289.5 nm,	-0.015
Max	287.3 nm,	0.007	Max	286.0 nm,	0.002
Min	284.1 nm,	-0.004	Max	282.3 nm,	0.007
Min	281.1 nm,	-0.009	Max	279.5 nm,	0.004
Min	275.7 nm,	-0.003	Max	274.8 nm,	0.003
Min	273.0 nm,	-0.004	Max	272.0 nm,	0.005
Min	268.5 nm,	-0.002	Max	267.5 nm,	0.005
Min	266.7 nm,	-0.003	Min	264.7 nm,	-0.001
Max	262.1 nm,	0.005			

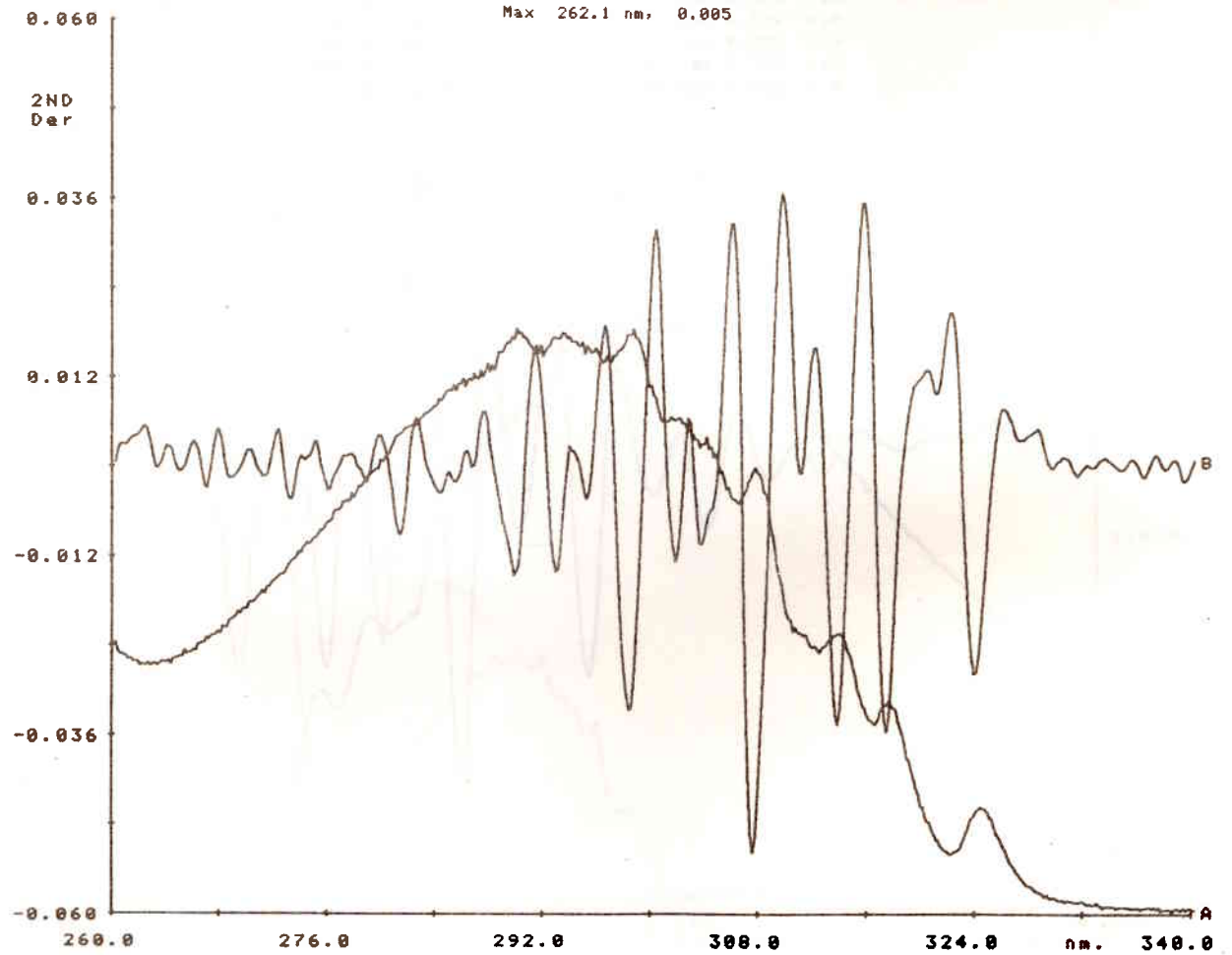

Figure 39

The corresponding 4th derivative spectrum (Figure 40), although enhancing peak resolution even more, shows more noise and requires greater care in its interpretation.

```
Trace   A                          Operator              Sample
Mode    4TH Der                    SBW (nm)    0.2       Smoothing (sec)   1
Ord Max/Min     0.006  -0.006      WL Max/Min (nm)  340.0   260.0
Speed (nm/min)    20               B'line Corr
    26 Peaks, threshold  0.005
Min  325.7 nm, -0.002   Max  323.7 nm,  0.004
Min  322.5 nm, -0.002   Max  317.0 nm,  0.004
Min  315.4 nm, -0.005   Max  313.6 nm,  0.004
Min  312.0 nm, -0.003   Max  310.6 nm,  0.004
Min  309.1 nm, -0.005   Min  307.9 nm,  0.000
Max  307.1 nm,  0.007   Min  305.5 nm, -0.005
Max  304.7 nm,  0.004   Min  304.0 nm, -0.001
Max  303.3 nm,  0.004   Min  302.7 nm, -0.005
Max  301.0 nm,  0.004   Min  300.1 nm, -0.006
Max  298.2 nm,  0.004   Min  296.2 nm, -0.004
Max  295.0 nm,  0.002   Min  293.6 nm, -0.004
Max  292.8 nm,  0.003   Min  291.0 nm, -0.004
Max  289.9 nm,  0.003   Min  272.1 nm, -0.003
```

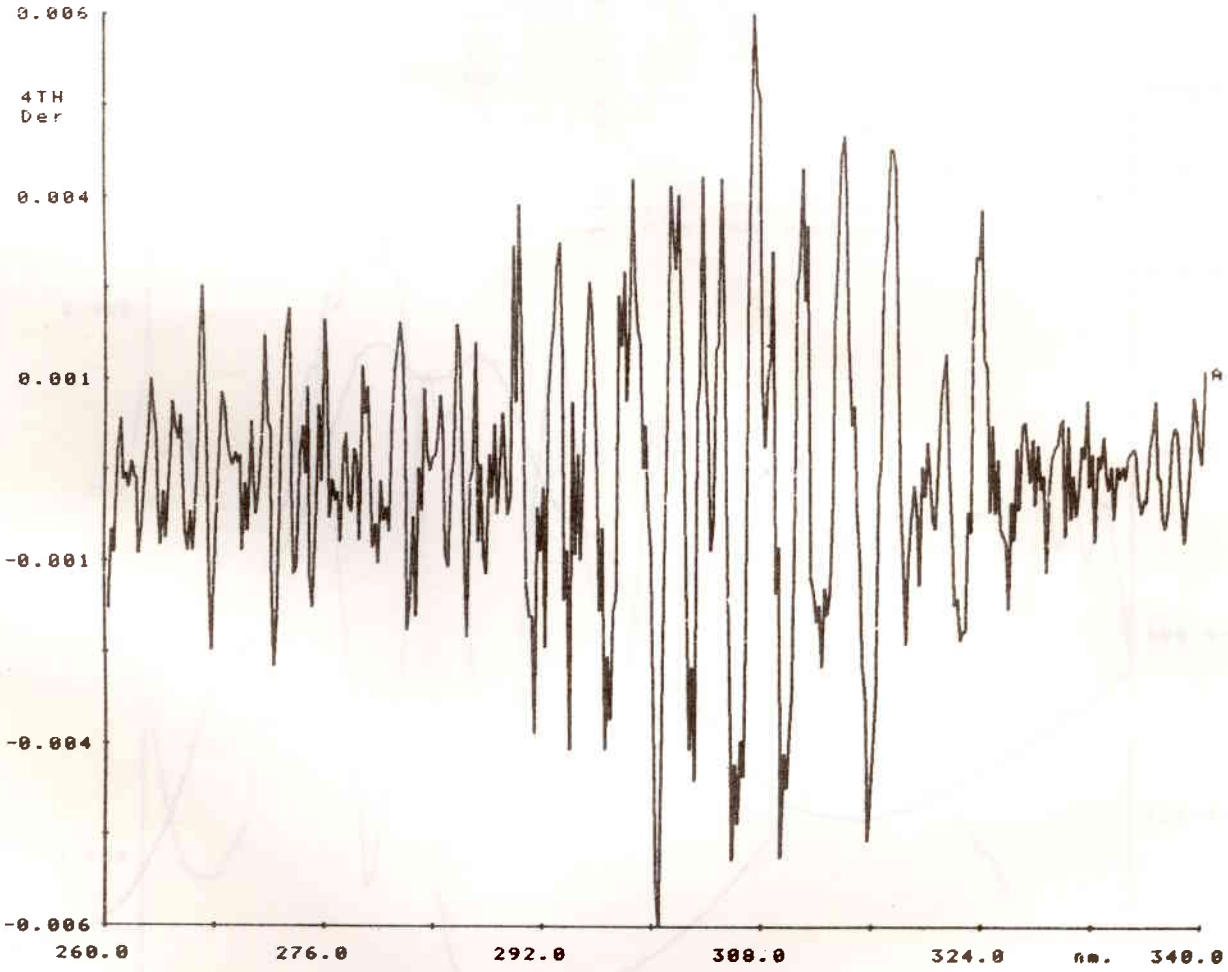

Figure 40

Similarly, the low UV peak envelope is readily resolved into the major peaks in the 2nd derivative spectrum (Figure 41).

```
Trace   A                          Operator            Sample
Mode    Abs                        SBW (nm)   0.2      Smoothing (sec)   0
Ord Max/Min    1.500   0.000       WL Max/Min (nm)  260.0   210.0
Speed (nm/min)    20
    32 Peaks, threshold  0.050
Min  259.7 nm,  0.076   Min  256.5 nm,  0.126
Min  255.0 nm,  0.192   Min  254.2 nm,  0.244
Min  252.5 nm,  0.419   Min  251.1 nm,  0.567
Min  250.5 nm,  0.620   Min  249.8 nm,  0.668
Min  248.8 nm,  0.715   Min  248.0 nm,  0.764
Min  247.1 nm,  0.841   Min  246.3 nm,  0.932
Max  244.2 nm,  1.074   Min  241.9 nm,  0.998
Max  239.9 nm,  1.054   Max  238.5 nm,  1.004
Max  237.9 nm,  0.953   Max  237.0 nm,  0.864
Max  236.2 nm,  0.814   Max  234.8 nm,  0.771
Max  233.5 nm,  0.717   Max  232.4 nm,  0.630
Max  231.5 nm,  0.552   Max  230.5 nm,  0.503
Max  228.9 nm,  0.452   Max  227.7 nm,  0.399
Max  226.5 nm,  0.344   Min  222.0 nm,  0.282
Min  218.3 nm,  0.330   Min  215.8 nm,  0.385
Min  213.7 nm,  0.432   Min  211.8 nm,  0.479
```

```
Trace   B                          Operator            Sample
Mode    2ND Der                    SBW (nm)   0.2      Smoothing (sec)   1
Ord Max/Min    0.040  -0.040       WL Max/Min (nm)  260.0   210.0
Speed (nm/min)    20
    23 Peaks, threshold  0.005
Min  258.4 nm,  0.002   Max  255.0 nm,  0.012
Max  253.6 nm,  0.010   Min  249.6 nm, -0.012
Max  247.3 nm,  0.015   Min  244.6 nm, -0.031
Max  242.0 nm,  0.014   Min  238.4 nm, -0.024
Max  236.1 nm,  0.019   Min  233.4 nm, -0.015
Max  231.4 nm,  0.011   Min  228.1 nm, -0.008
Max  226.3 nm,  0.008   Min  223.1 nm, -0.001
Max  221.8 nm,  0.005   Min  218.6 nm, -0.001
Max  215.8 nm,  0.004   Min  214.8 nm, -0.007
Max  213.8 nm,  0.005   Min  213.2 nm, -0.001
Max  212.5 nm,  0.004   Min  211.8 nm, -0.003
Max  211.1 nm,  0.003
```

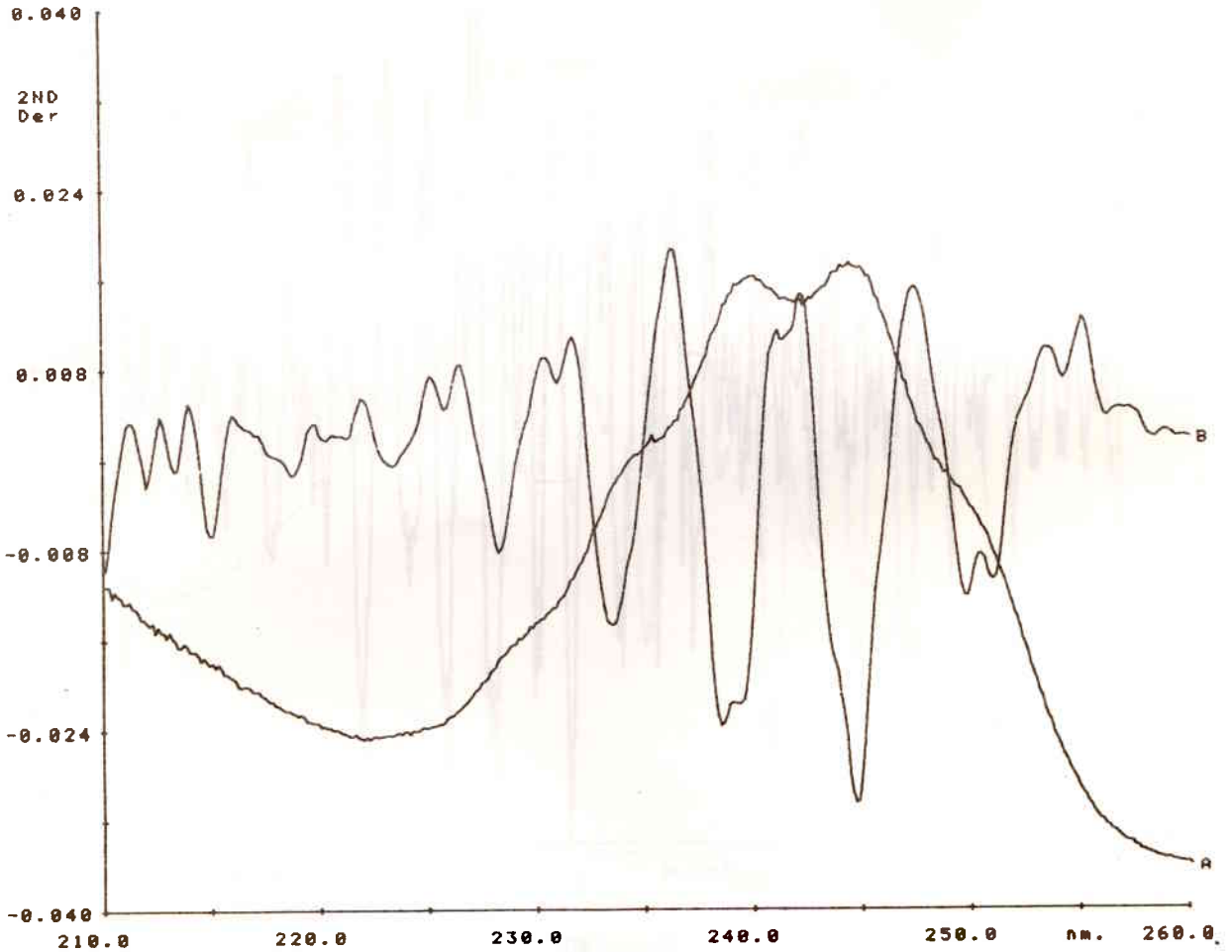

Figure 41

In the third diazine isomer, pyridazine, the less intense peak envelope is quite significantly shifted to longer wavelengths (Figure 42).

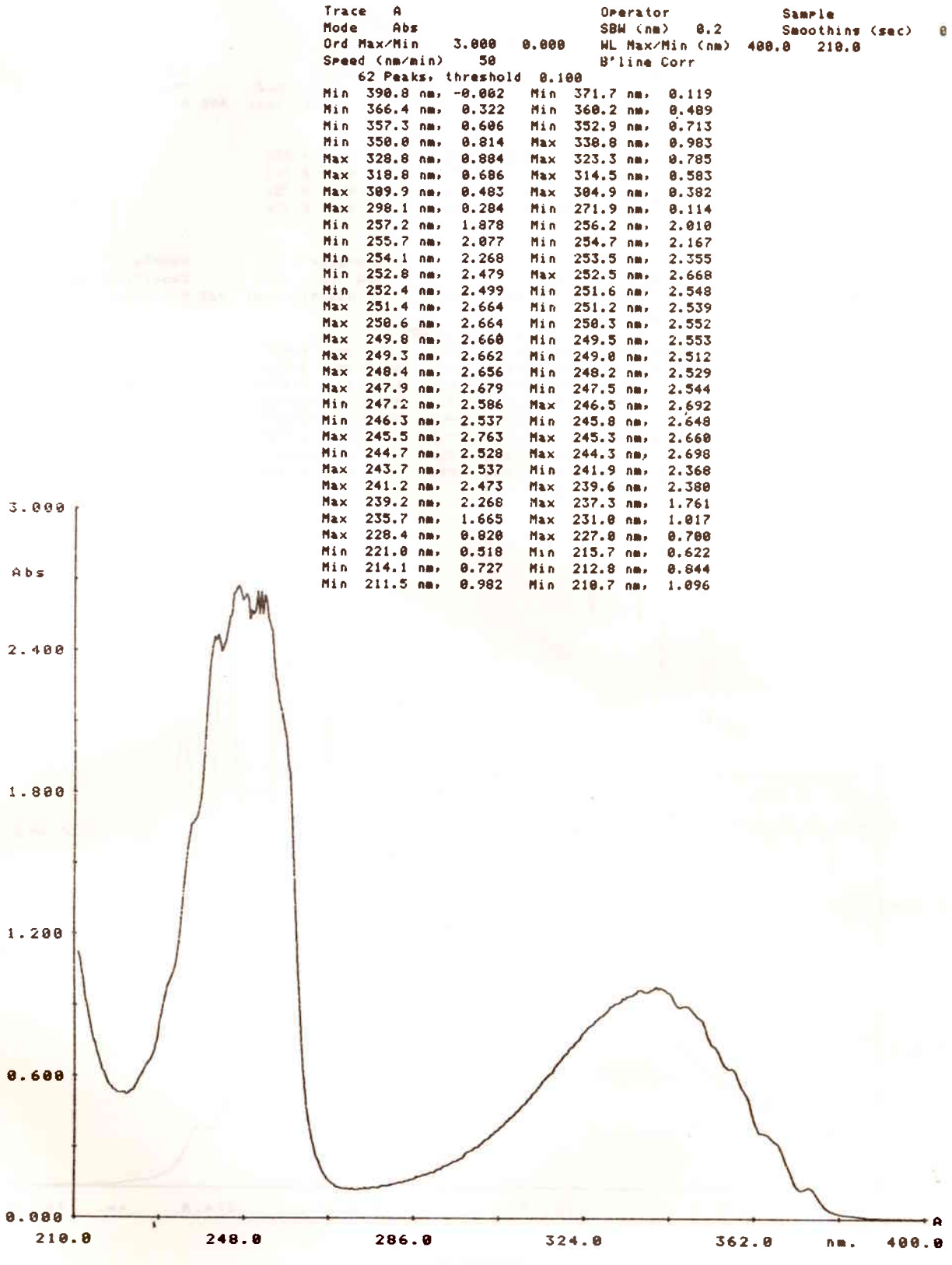

Figure 42

The resolution obtained in the 2nd order derivative spectrum (Figure 43) hints at a very complex peak structure, which is further resolved in the 4th order derivative spectrum (Figure 44), where the less intense peak positions can be obtained by the 'zoom' facility.

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   0.2        Smoothing (sec)   0
Ord Max/Min     1.500    0.000     WL Max/Min (nm)  400.0   270.0
Speed (nm/min)     50
     8 Peaks, threshold  0.200
Min  390.8 nm, -0.002    Min  365.4 nm,  0.338
Min  358.9 nm,  0.544    Min  350.8 nm,  0.763
Max  340.1 nm,  0.985    Max  323.0 nm,  0.782
Max  314.4 nm,  0.581    Max  304.6 nm,  0.376
```

```
Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   0.2        Smoothing (sec)   1
Ord Max/Min     0.025   -0.025     WL Max/Min (nm)  400.0   270.0
Speed (nm/min)     50
    17 Peaks, threshold  0.005
Min  382.7 nm, -0.001    Max  376.2 nm,  0.007
Min  373.2 nm, -0.012    Max  369.9 nm,  0.016
Min  365.9 nm, -0.011    Max  361.2 nm,  0.020
Max  357.5 nm, -0.005    Min  356.0 nm, -0.011
Max  353.8 nm,  0.008    Min  352.1 nm, -0.001
Max  350.4 nm,  0.006    Min  348.3 nm, -0.009
Max  343.1 nm,  0.010    Min  339.6 nm, -0.009
Max  336.5 nm,  0.003    Max  332.5 nm,  0.000
Min  328.6 nm, -0.005
```

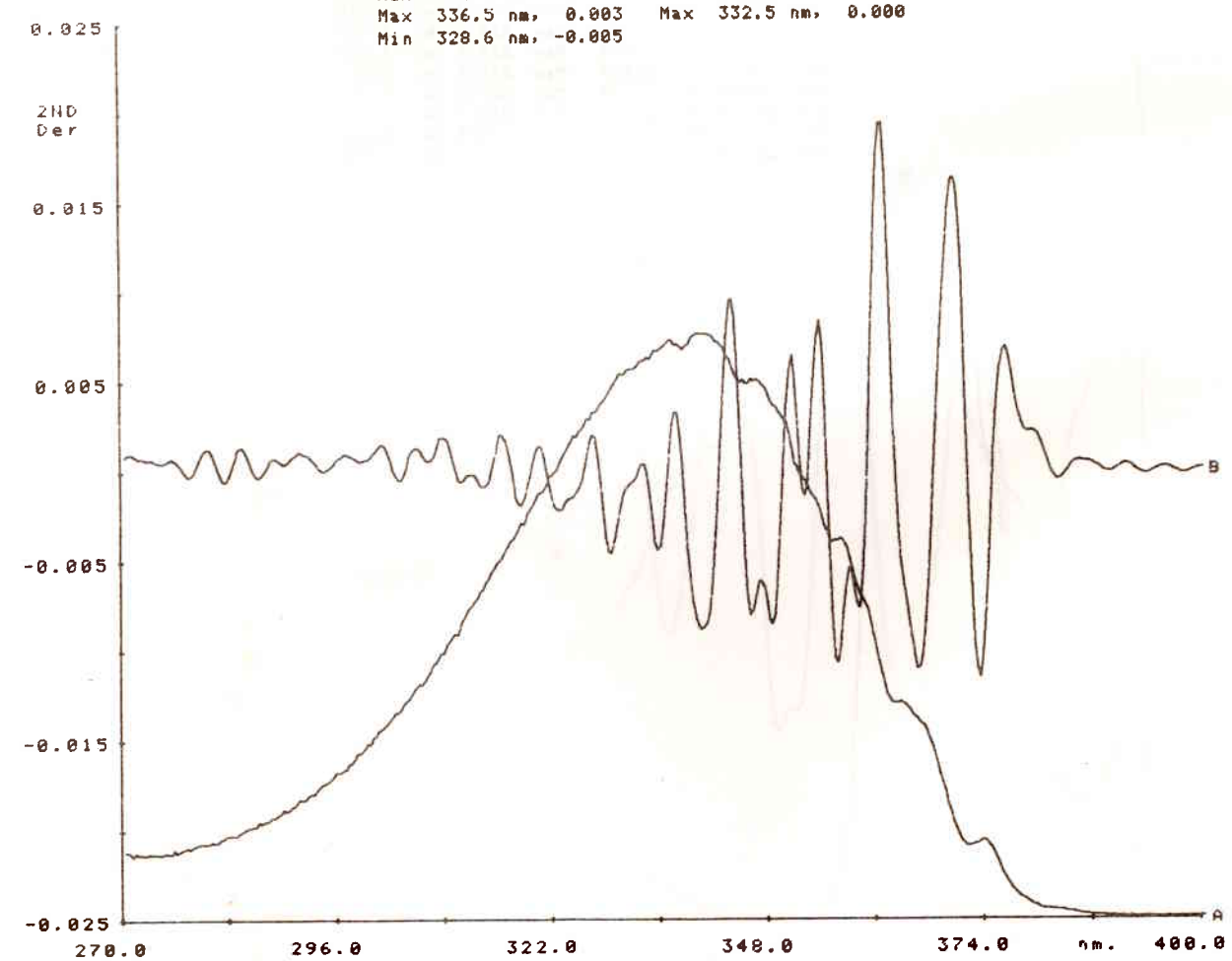

Figure 43

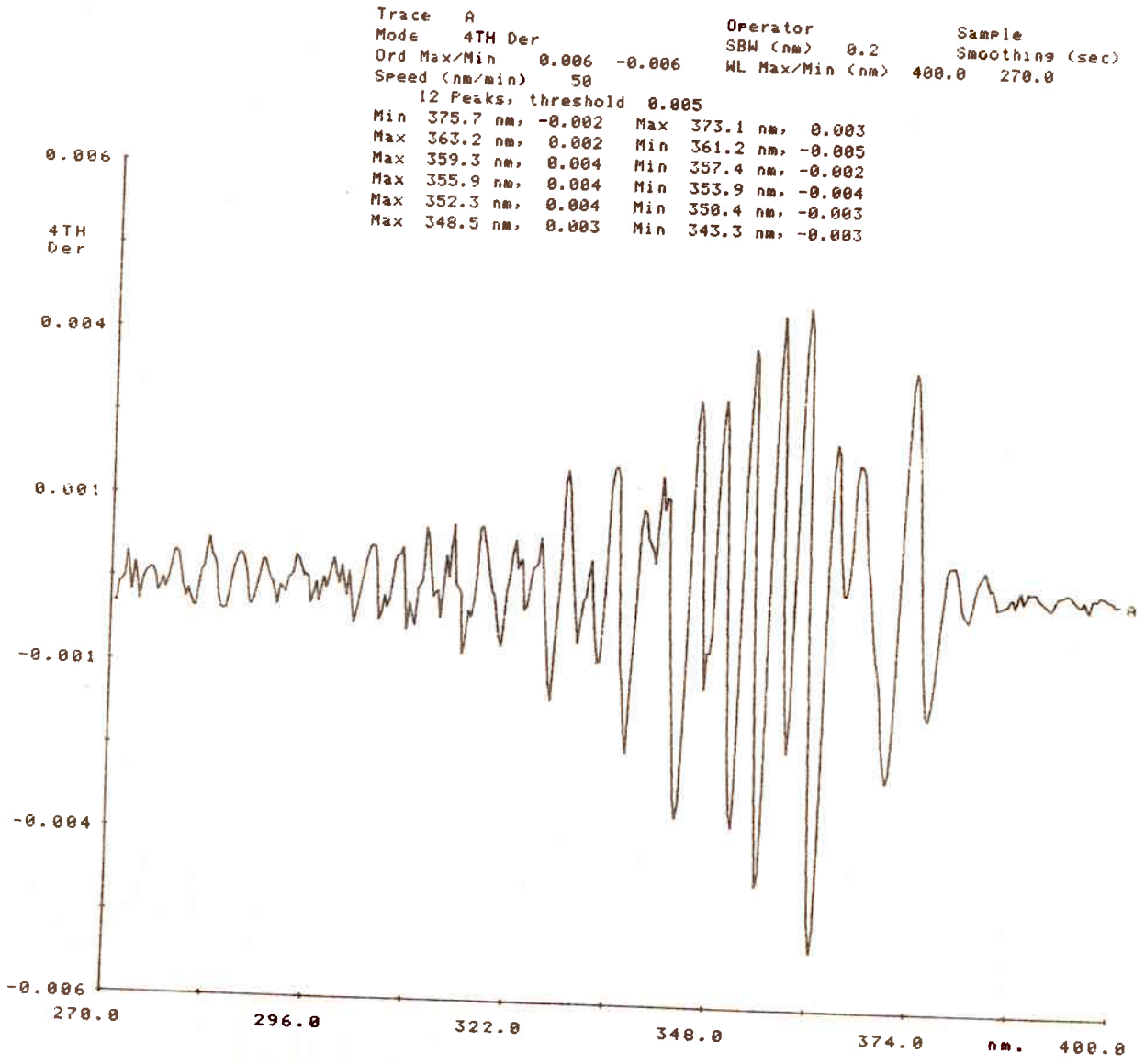
Trace A
Mode 4TH Der
Ord Max/Min 0.006 -0.006
Speed (nm/min) 50
Operator
SBW (nm) 0.2
WL Max/Min (nm) 400.0 270.0
Sample
Smoothing (sec)
12 Peaks, threshold 0.005
Min 375.7 nm, -0.002
Max 373.1 nm, 0.003
Max 363.2 nm, 0.002
Min 361.2 nm, -0.005
Max 359.3 nm, 0.004
Min 357.4 nm, -0.002
Max 355.9 nm, 0.004
Min 353.9 nm, -0.004
Max 352.3 nm, 0.004
Min 350.4 nm, -0.003
Max 348.5 nm, 0.003
Min 343.3 nm, -0.003
0.006
4TH
Der
0.004
0.001
-0.001
-0.004
-0.006
270.0
296.0
322.0
348.0
374.0
nm.
400.0
A

Figure 44

The short wavelength peak envelope can be likewise characterized by going to higher derivatives (Figure 45 and Figure 46).

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   0.2        Smoothing (sec)   0
Ord Max/Min     2.500    0.000     WL Max/Min (nm)  270.0   210.0
Speed (nm/min)     50             B'line Corr
    11 Peaks, threshold  0.200
Min  269.8 nm,  0.076   Min  261.3 nm,  0.277
Min  255.8 nm,  1.241   Max  251.2 nm,  1.977
Min  248.3 nm,  1.743   Max  246.2 nm,  1.996
Max  243.1 nm,  1.515   Max  237.7 nm,  1.018
Max  231.9 nm,  0.585   Min  220.5 nm,  0.282
Min  212.3 nm,  0.496

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   0.2        Smoothing (sec)   1
Ord Max/Min     0.150   -0.150     WL Max/Min (nm)  270.0   210.0
Speed (nm/min)     50             B'line Corr
    16 Peaks, threshold  0.005
Max  259.5 nm,  0.057   Min  256.1 nm, -0.057
Max  253.2 nm,  0.053   Min  250.3 nm, -0.113
Max  247.5 nm,  0.073   Min  244.7 nm, -0.111
Max  241.9 nm,  0.071   Min  239.2 nm, -0.067
Max  236.3 nm,  0.051   Min  233.8 nm, -0.027
Max  231.2 nm,  0.029   Min  228.8 nm, -0.006
Max  226.3 nm,  0.011   Min  223.8 nm,  0.001
Min  219.4 nm,  0.004   Max  211.6 nm,  0.014
```

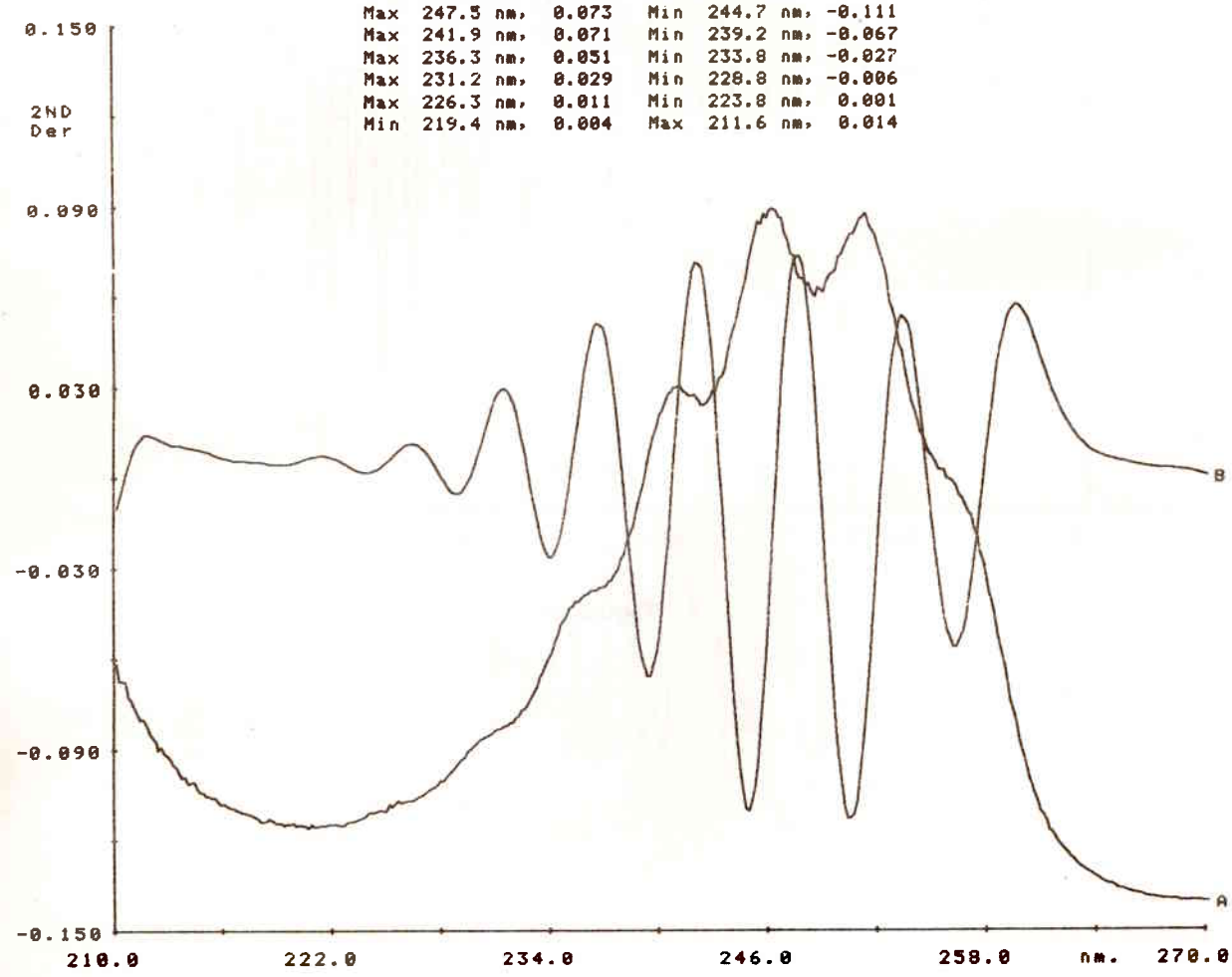

Figure 45

Trace A		Operator	Sample
Mode 2ND Der		SBW (nm) 0.2	Smoothing (sec) 1
Ord Max/Min	0.100 -0.100	WL Max/Min (nm)	270.0 210.0
Speed (nm/min)	50	B'line Corr	

16 Peaks, threshold 0.005

Max	259.5 nm,	0.096	Min	256.1 nm,	-0.103
Max	253.5 nm,	0.010	Min	251.1 nm,	-0.048
Max	246.6 nm,	0.042	Min	244.2 nm,	-0.083
Max	241.7 nm,	0.039	Min	239.2 nm,	-0.078
Max	236.3 nm,	0.065	Min	233.8 nm,	-0.048
Max	231.3 nm,	0.051	Min	228.7 nm,	-0.011
Max	226.3 nm,	0.022	Min	223.9 nm,	0.002
Min	219.4 nm,	0.008	Max	212.0 nm,	0.024

Trace B		Operator	Sample
Mode 4TH Der		SBW (nm) 0.2	Smoothing (sec) 1
Ord Max/Min	0.040 -0.040	WL Max/Min (nm)	270.0 210.0
Speed (nm/min)	50	B'line Corr	

22 Peaks, threshold 0.005

Max	262.0 nm,	0.003	Min	259.0 nm,	-0.016
Max	256.5 nm,	0.024	Max	255.7 nm,	0.019
Min	253.9 nm,	-0.016	Min	252.3 nm,	-0.001
Max	251.3 nm,	0.013	Min	250.2 nm,	-0.004
Min	249.1 nm,	-0.000	Max	248.2 nm,	0.012
Min	246.6 nm,	-0.022	Max	244.2 nm,	0.030
Min	242.1 nm,	-0.023	Max	239.1 nm,	0.019
Min	236.4 nm,	-0.023	Max	233.8 nm,	0.020
Min	231.4 nm,	-0.014	Max	228.9 nm,	0.008
Min	226.4 nm,	-0.005	Max	224.0 nm,	0.003
Max	214.1 nm,	0.002	Min	211.4 nm,	-0.008

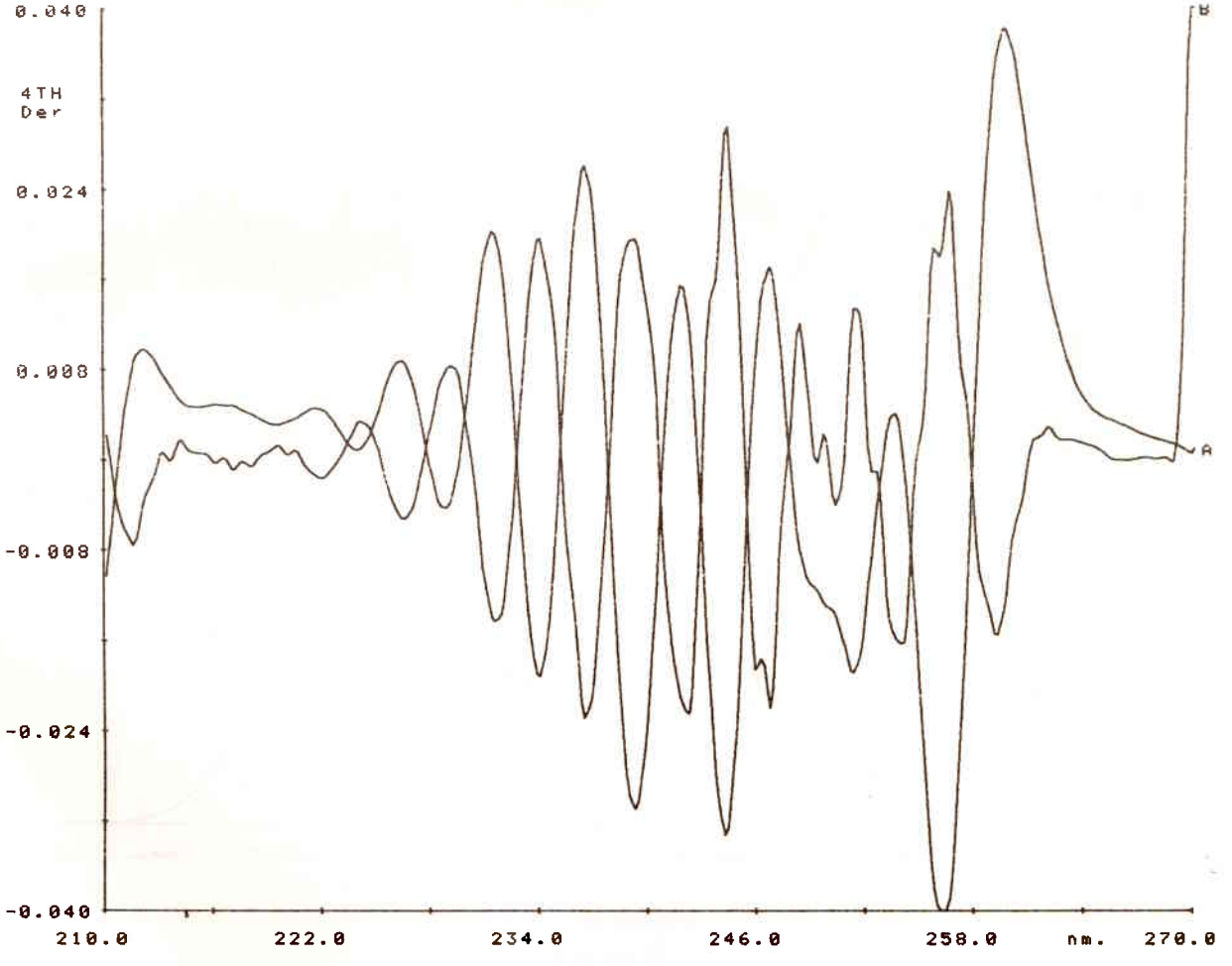

Figure 46

These three examples clearly illustrate the potential usefulness of derivative techniques for the re-investigation of the UV-Visible spectra of many compounds, and not only members of homologous or isomeric series.

Quantitative Determination of Trace Compounds

The ability to locate hidden peaks in a spectrum of overlapping peaks makes the derivative technique of particular interest for the quantitation of trace components in complex matrices.

For example, the spectrum of caffeine (1,3,7-trimethylxanthine) in water shows two fairly broad peaks at about 273 nm and 204 nm, together with a prominent shoulder between these two peaks (Figure 47).

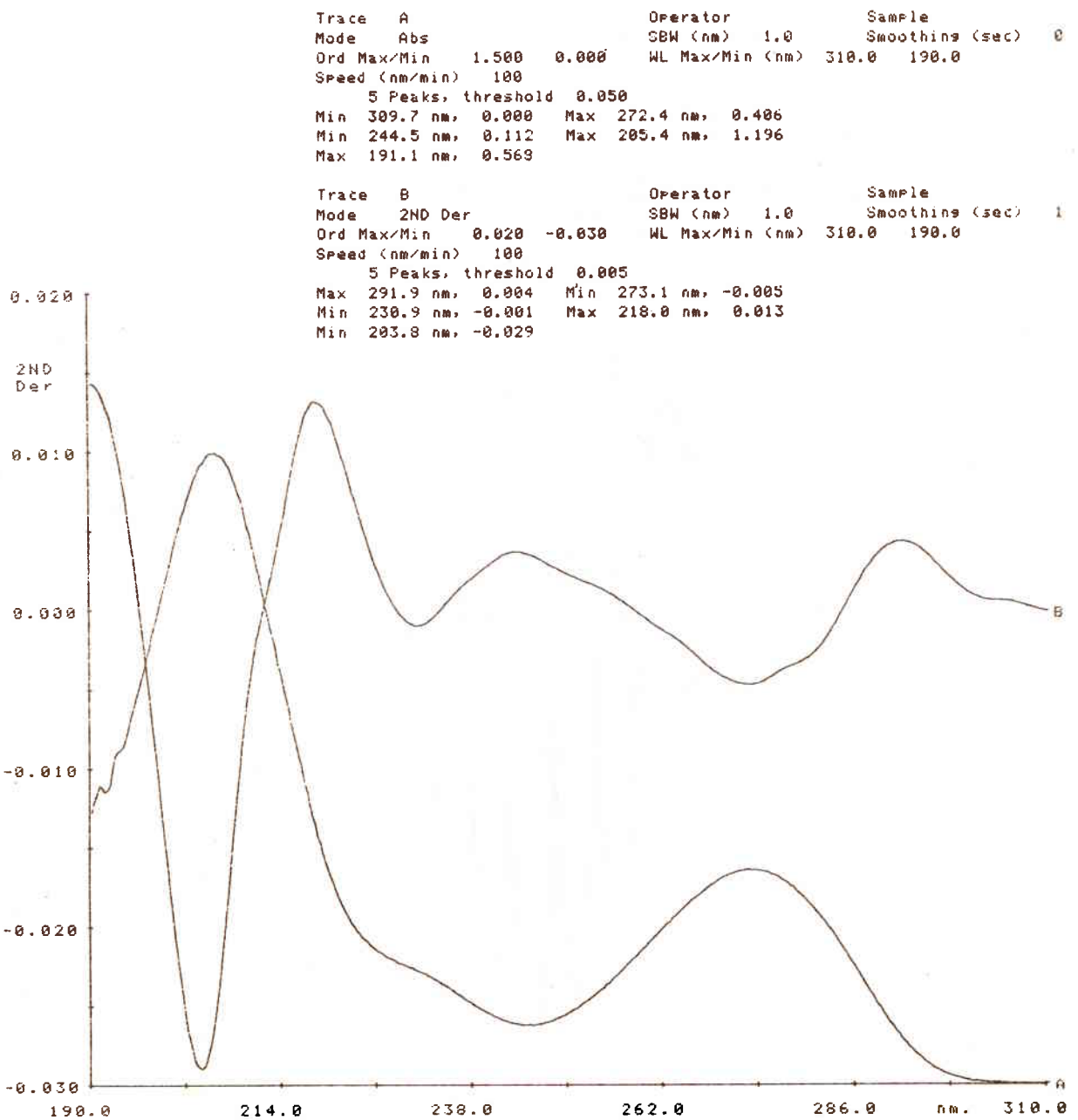

Figure 47

The 2nd derivative shows this shoulder to be at about 231 nm.

Caffeine occurs widely in natural products as well as in some commercial products such as, for example, COCA-COLA and PEPSI-COLA.

The UV zero order spectra and the derivative spectra of these COLA's (degassed and diluted 50 times) bear a close resemblance to the pure caffeine spectra. Thus, in the COCA-COLA spectra (Figure 48) and in the PEPSI-COLA spectra (Figure 49) the three peaks occur at about 276-278 nm, 230 nm and 205 nm.

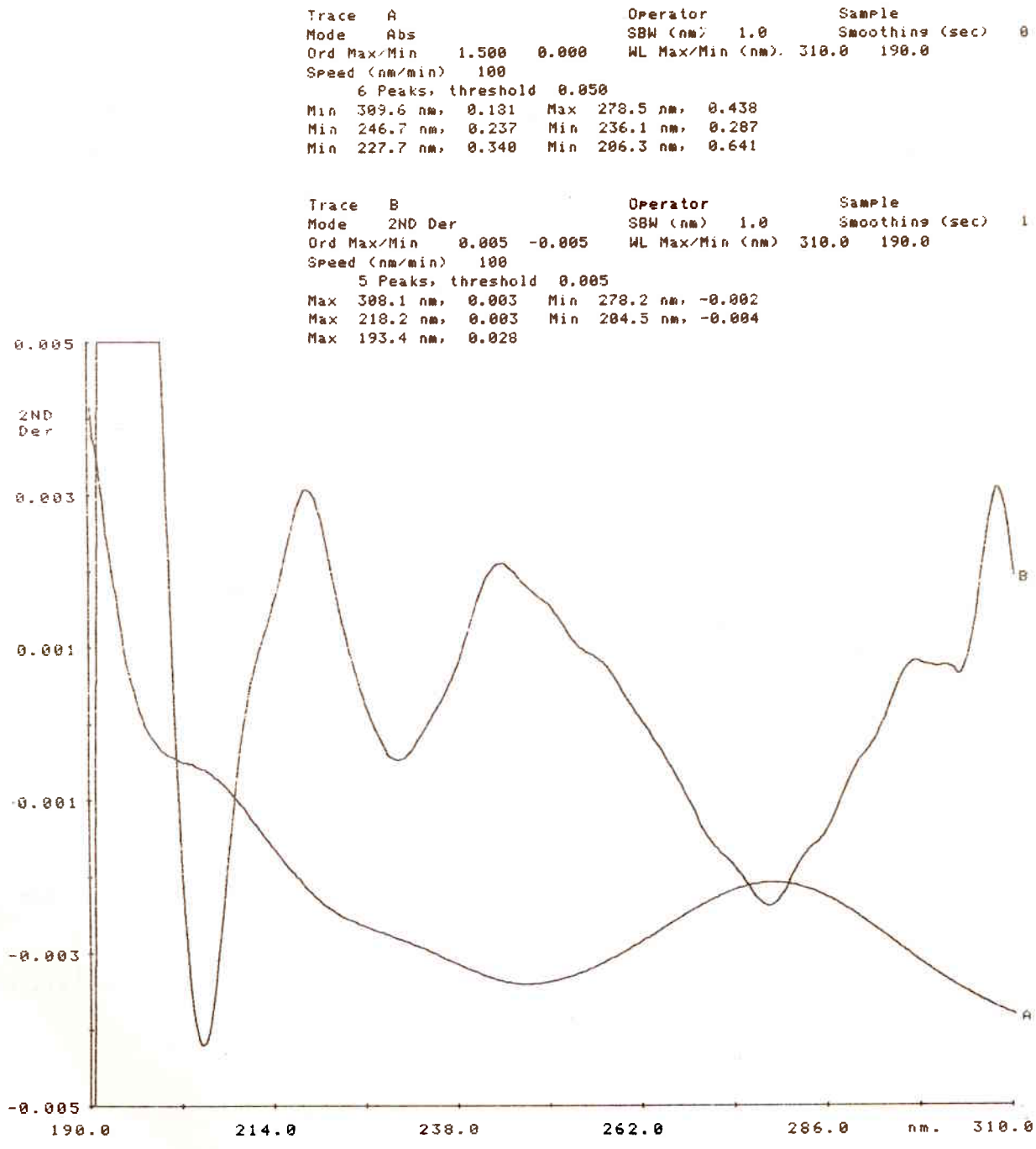

Figure 48

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   1.0        Smoothing (sec)   0
Ord Max/Min    1.500    0.000      WL Max/Min (nm)  310.0   190.0
Speed (nm/min)   100              B'line Corr
     8 Peaks, threshold  0.050
Min  309.4 nm,  0.119   Min  289.8 nm,  0.181
Max  274.2 nm,  0.240   Min  248.8 nm,  0.166
Min  232.5 nm,  0.236   Min  223.3 nm,  0.298
Min  210.7 nm,  0.467   Min  206.3 nm,  0.528

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   1.0        Smoothing (sec)   1
Ord Max/Min    0.005   -0.005      WL Max/Min (nm)  310.0   190.0
Speed (nm/min)   100
     3 Peaks, threshold  0.005
Max  219.2 nm,  0.002   Min  205.0 nm, -0.003
Max  193.9 nm,  0.030
```

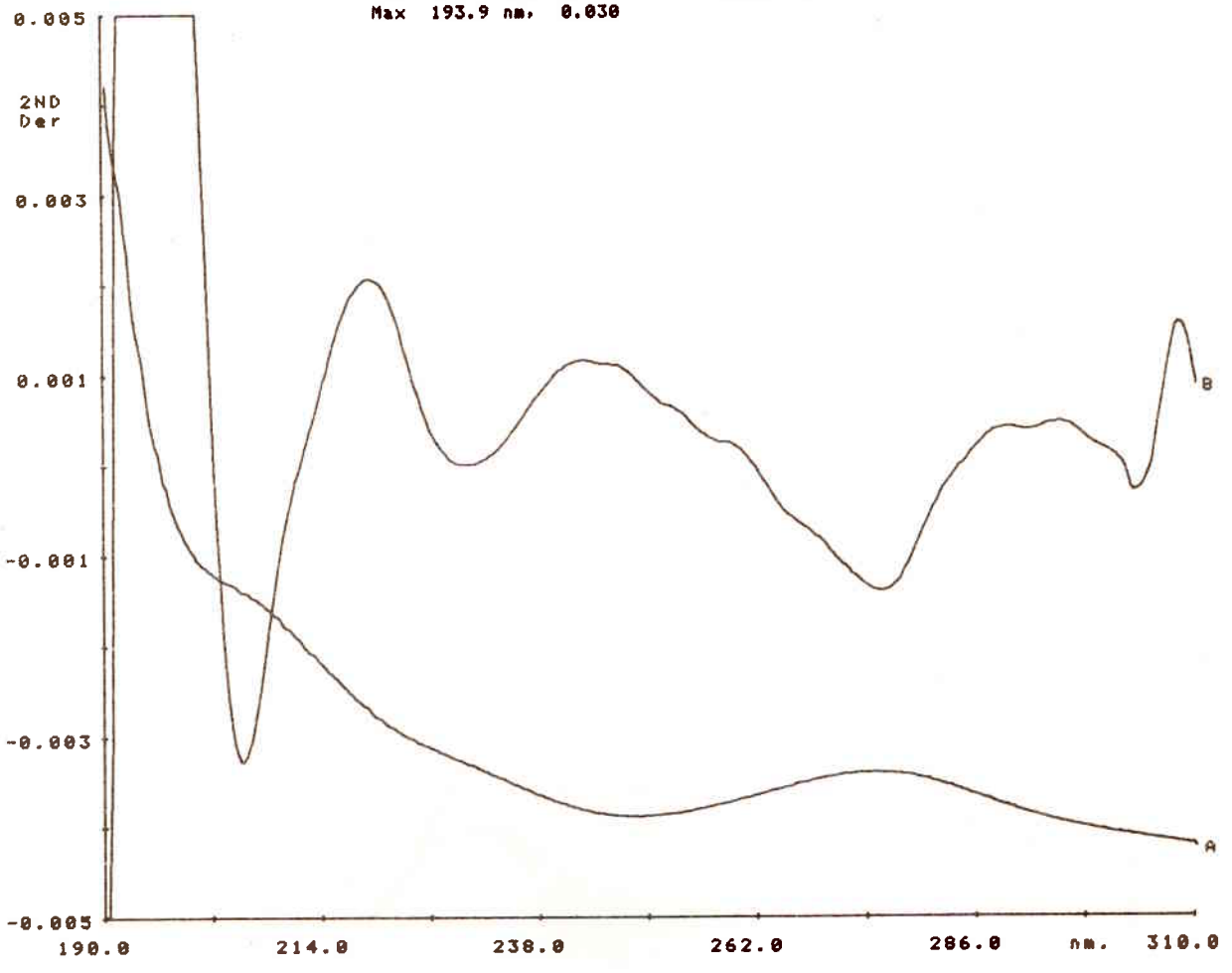

Figure 49

Using the method of standard additions and measuring the 2nd derivative peak amplitudes, D_L, of the 273 nm peak (Figure 50) linear calibrations were obtained (Figure 51) which gave calculated values for caffeine of about 100 mg/l in PEPSI-COLA and 300 mg/l in COCA-COLA.

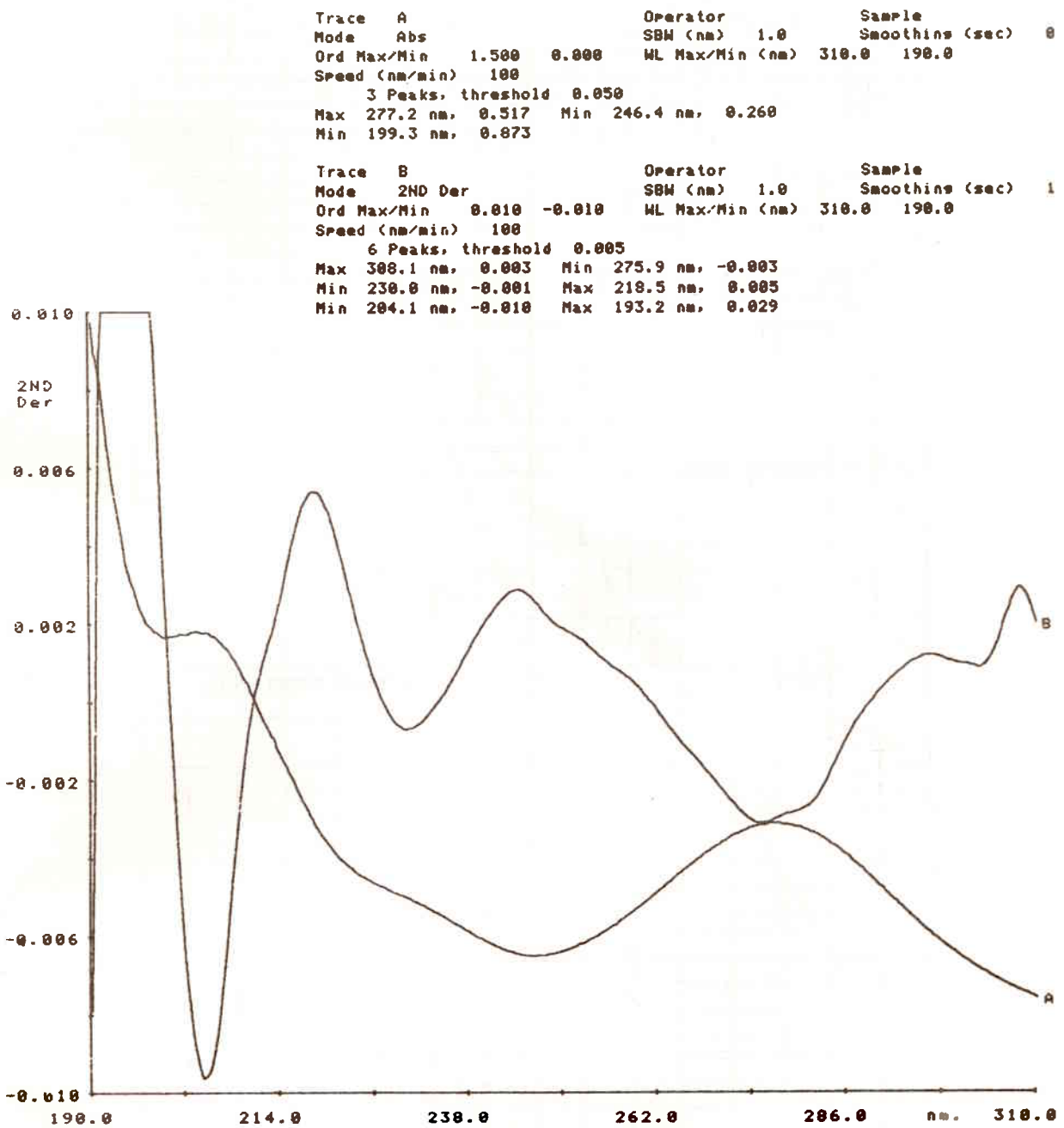

Figure 50

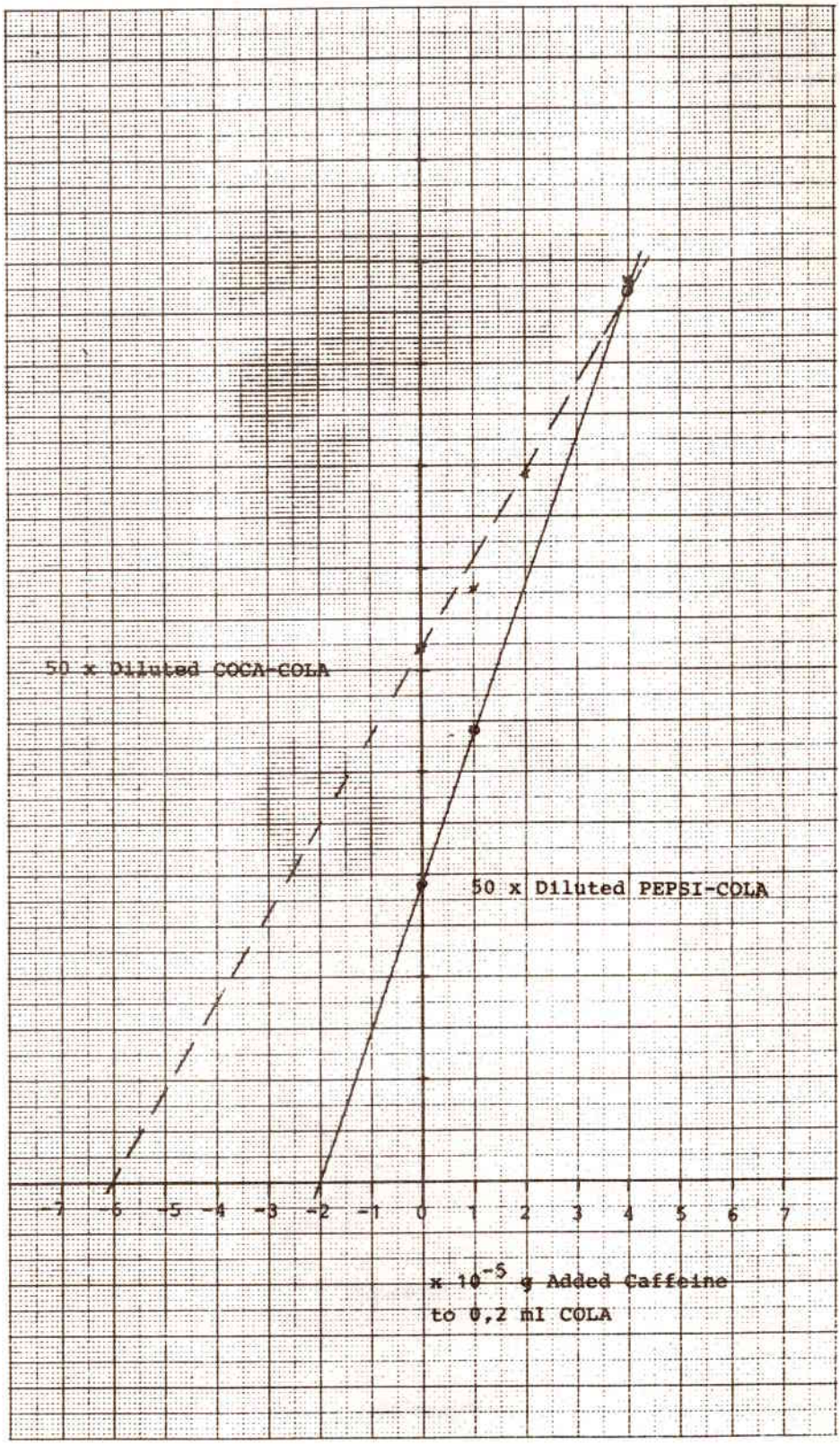
50 x Diluted COCA-COLA
50 x Diluted PEPSI-COLA
-7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7
x 10^-5 g Added Caffeine
to 0,2 ml COLA

Figure 51

Of course, the other two wavelength peak amplitudes, as well as the zero-line, D_Z, and short wavelength side, D_S, amplitudes may also be used for calibrations and concentration calculations, if they are appropriately linear over the concentration range of interest.

This, by no means exhaustive investigation, shows the potential usefulness of derivative peak amplitudes for the quantitation of both major and minor constituents in natural and commercial materials.

As discussed in Part I, higher derivative techniques can be used to advantage to eliminate completely or at least to minimize undesirable, interfering background which may be present due to matrix absorption or scattering.

For example, the spectrum of a dilute acid solution of the lanthanide, holmium, shows no significant background in the region 235-260 nm, so that the holmium absorption peak at about 241 nm can be readily quantified, with or without the aid of derivative spectroscopy (Figure 52).

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   0.3        Smoothing (sec)   0
Ord Max/Min    4.000    0.000      WL Max/Min (nm)  260.0   235.0
Speed (nm/min)    20
    20 Peaks, threshold  0.100
Min  259.5 nm,  0.805   Min  254.8 nm,  0.906
Min  251.9 nm,  1.023   Min  249.0 nm,  1.134
Min  246.9 nm,  1.234   Min  245.3 nm,  1.341
Min  244.0 nm,  1.485   Max  241.7 nm,  2.120
Min  241.0 nm,  1.882   Min  239.9 nm,  1.983
Min  238.8 nm,  2.173   Min  237.8 nm,  2.376
Min  237.2 nm,  2.463   Min  236.9 nm,  2.560
Min  236.4 nm,  2.683   Min  235.8 nm,  2.771
Max  235.6 nm,  2.967   Min  235.5 nm,  2.856
Min  235.2 nm,  2.863   Min  235.1 nm,  2.967

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   0.3        Smoothing (sec)   1
Ord Max/Min    0.300   -0.300      WL Max/Min (nm)  260.0   235.0
Speed (nm/min)    20
    5 Peaks, threshold  0.100
Min  250.6 nm, -0.014   Max  242.3 nm,  0.114
Min  241.3 nm, -0.243   Max  240.3 nm,  0.129
Min  235.9 nm, -0.054

Trace   C                          Operator              Sample
Mode    4TH Der                    SBW (nm)   0.3        Smoothing (sec)   1
Ord Max/Min    0.100   -0.100      WL Max/Min (nm)  260.0   235.0
Speed (nm/min)    20
    2 Peaks, threshold  0.100
Min  242.1 nm, -0.060   Max  241.3 nm,  0.084
```

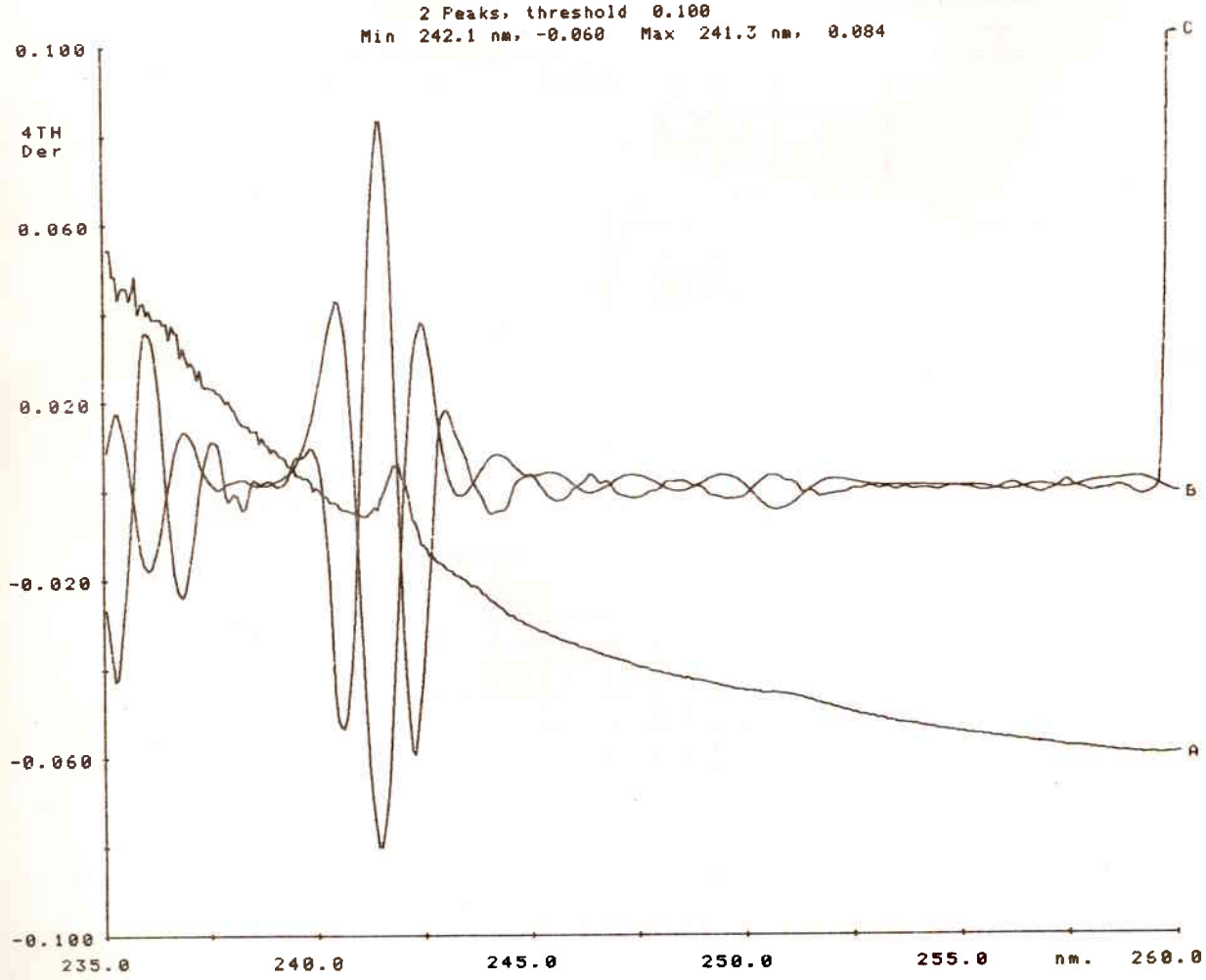

Figure 52

However, the spectrum of a holmium glass filter in this region shows a severely increasing background towards shorter wavelengths. (Figure 53)

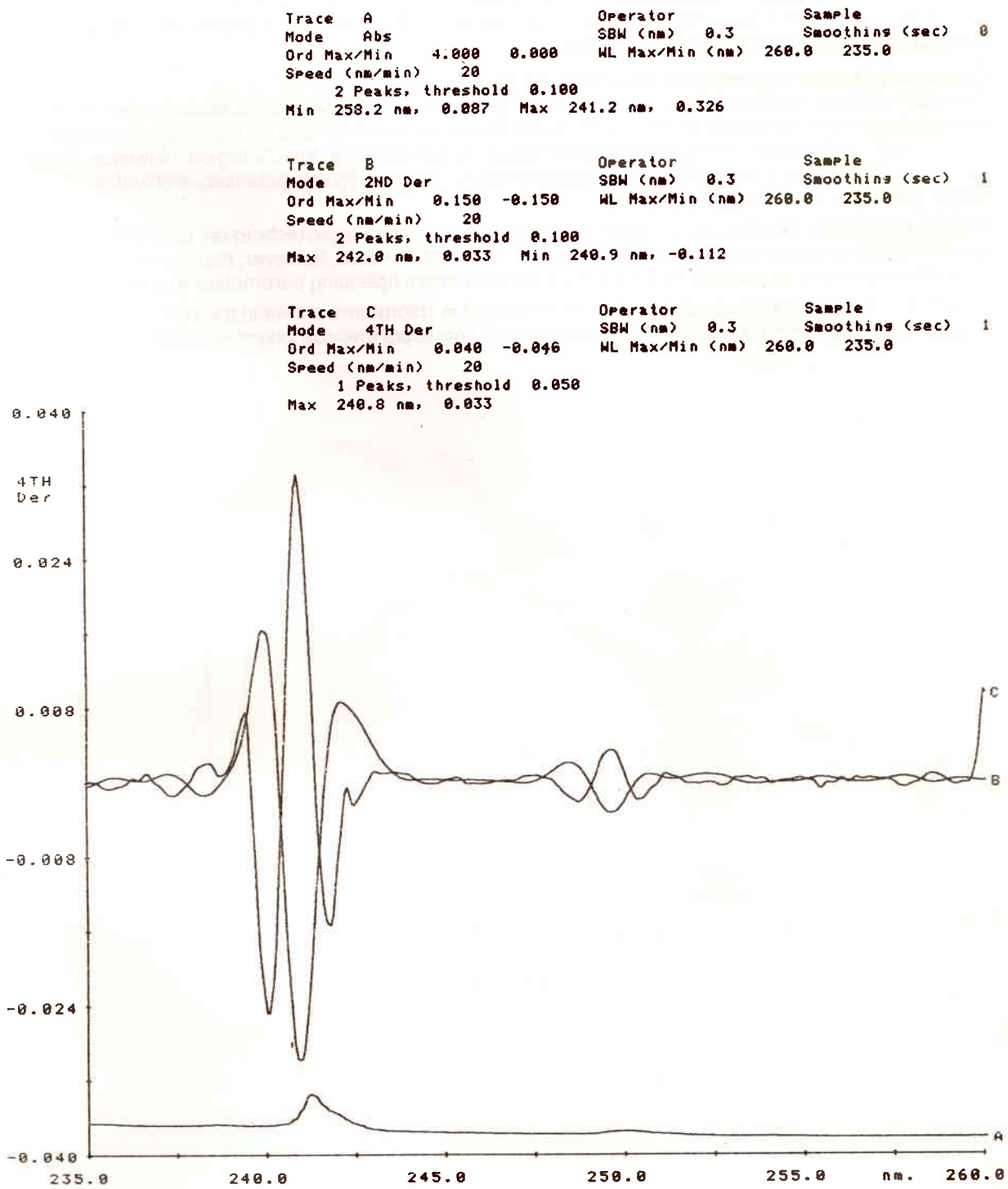

Figure 53

This background is completely eliminated already in the 2nd order derivative spectrum, so that in this case there is no particular need to go to the 4th or higher order derivatives in order to quantify the peak at 241 nm.

In the author's opinion, in background absorption or scattering situations the use of derivative spectroscopy is very often much simpler and more safe to use than the more commonly used 3- or 2-point correction techniques, which are based on the assumption that over a short wavelength range the change in background absorption is linear.

Characterization of Commercial Materials and Natural Substances

The unambiguous characterization of most natural products and commercial materials by the UV-Visible spectrophotometric technique alone is seldom completely successful, because most substances exhibit rather broad, featureless, non-specific absorption bands, particularly in the UV region. However, higher derivative spectroscopy does offer greater possibilities to obtain more characteristic, archivable 'finger-print' spectra of many substances.

In order to highlight the potential of higher order derivative spectroscopic techniques for finger-printing, several readily available materials were measured. It should be noted, however, that in this preliminary illustrative survey no exhaustive optimization of the instrument operating parameters was attempted.

A sample of a commercially available olive oil, dissolved in chloroform, shows in the zero order spectrum (Figure 54) a single peak at 243 nm and several broad shoulders towards longer wavelengths.

```
Trace   A                              Operator              Sample
Mode    Abs                            SBW (nm)   1.0        Smoothing (sec)   0
Ord Max/Min     2.000    0.000         WL Max/Min (nm)  350.0    220.0
Speed (nm/min)    100
     6 Peaks, threshold  0.050
Min  349.4 nm,  0.017    Min  303.2 nm,  0.086
Min  282.6 nm,  0.246    Max  243.1 nm,  1.542
Max  235.2 nm,  0.455    Max  232.1 nm,  0.409
```

```
Trace   B                              Operator              Sample
Mode    2ND Der                        SBW (nm)   1.0        Smoothing (sec)   1
Ord Max/Min     0.100   -0.100         WL Max/Min (nm)  350.0    220.0
Speed (nm/min)    100
     5 Peaks, threshold  0.005
Min  284.6 nm, -0.003    Max  254.7 nm,  0.017
Min  240.3 nm, -0.099    Max  234.9 nm,  0.071
Max  224.8 nm,  0.006
```

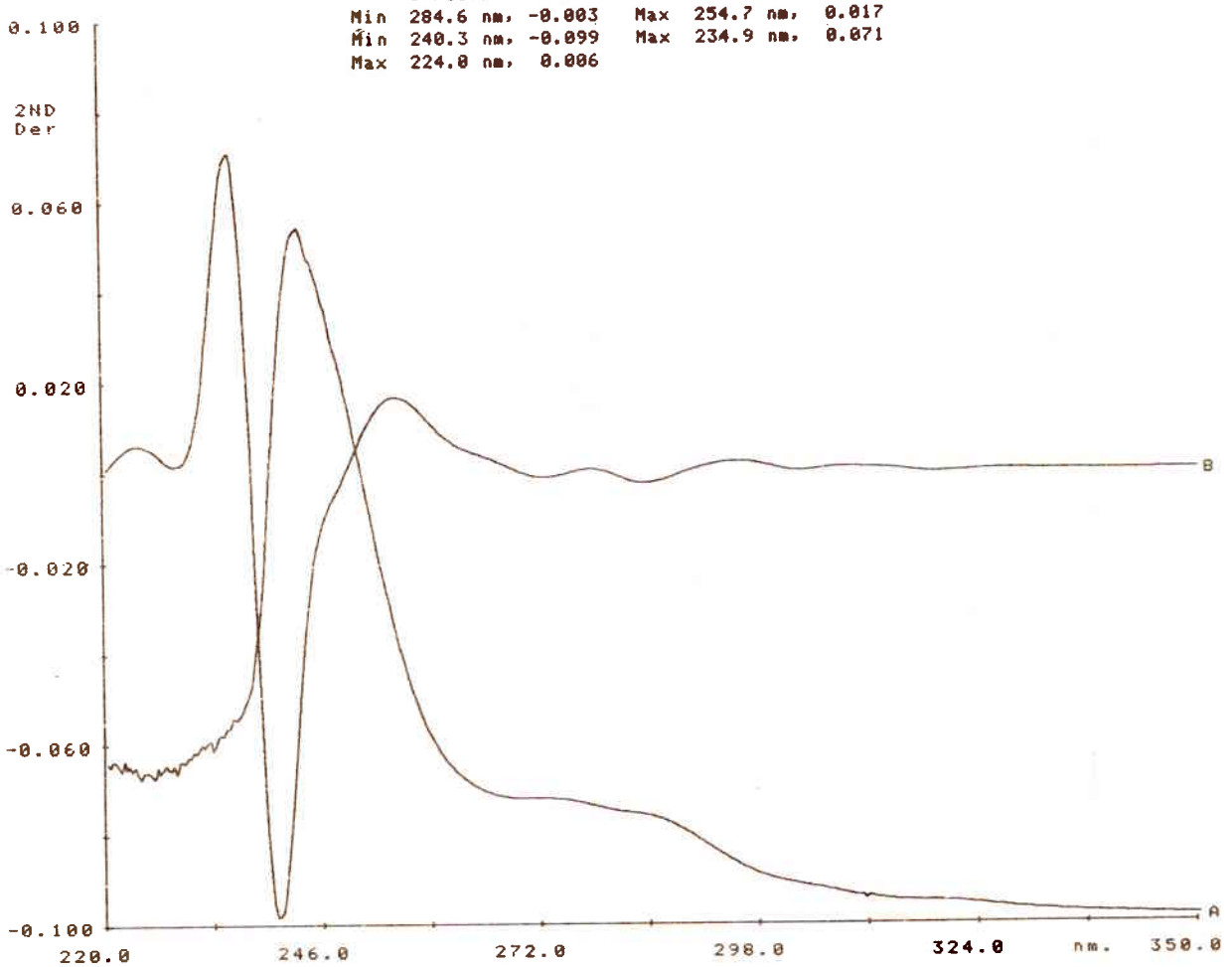

Figure 54

These shoulders are easily resolved in the 2nd order derivative spectrum into four quite distinctive peaks (Figure 55), which could be useful for either characterization or comparison purposes.

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   1.0        Smoothing (sec)   0
Ord Max/Min    2.000    0.000      WL Max/Min (nm)  350.0   220.0
Speed (nm/min)   100
     6 Peaks, threshold  0.050
Min  349.4 nm,  0.017   Min  303.2 nm,  0.086
Min  282.6 nm,  0.246   Max  243.1 nm,  1.542
Max  235.2 nm,  0.455   Max  232.1 nm,  0.409

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   1.0        Smoothing (sec)   1
Ord Max/Min    0.005  -0.005      WL Max/Min (nm)  350.0   220.0
Speed (nm/min)   100
     5 Peaks, threshold  0.005
Min  284.6 nm, -0.003   Max  254.7 nm,  0.017
Min  240.3 nm, -0.099   Max  234.9 nm,  0.071
Max  224.0 nm,  0.006
```

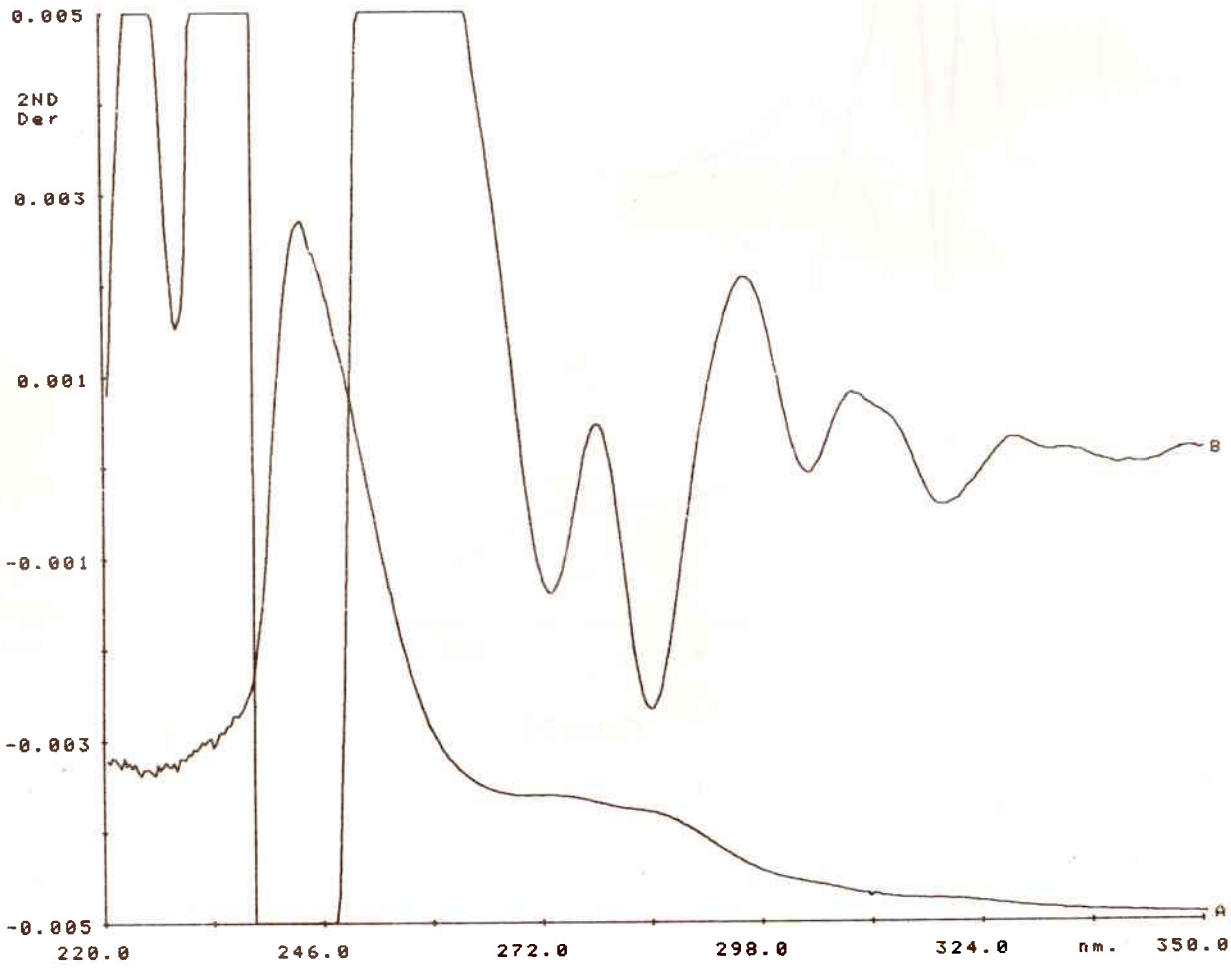

Figure 55

A commercial material such as a perfume or aftershave lotion is, of course, a very complex mixture of substances with extensive overlapping of absorption bands, which only the manufacturer is able to characterize easily. Nevertheless, it is possible to resolve some of the overlapping peaks with derivative techniques, as shown in the following spectrum of an aftershave lotion (Figure 56).

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)    1.0       Smoothing (sec)   0
Ord Max/Min     2.500    0.000     WL Max/Min (nm)  350.0    200.0
Speed (nm/min)    100
     5 Peaks, threshold  0.100
Min  348.7 nm,  0.042   Max  281.1 nm,  1.005
Min  249.1 nm,  0.539   Min  210.0 nm,  2.200
Max  206.8 nm,  2.469

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)    1.0       Smoothing (sec)   1
Ord Max/Min     0.150   -0.150     WL Max/Min (nm)  350.0    200.0
Speed (nm/min)    100
     6 Peaks, threshold  0.005
Max  312.4 nm,  0.004   Min  283.7 nm, -0.005
Max  238.0 nm,  0.008   Min  225.2 nm, -0.010
Max  214.1 nm,  0.014   Min  203.5 nm, -0.115

Trace   C                          Operator              Sample
Mode    2ND Der                    SBW (nm)    1.0       Smoothing (sec)   1
Ord Max/Min     0.010   -0.010     WL Max/Min (nm)  350.0    200.0
Speed (nm/min)    100
     6 Peaks, threshold  0.005
Max  312.4 nm,  0.004   Min  283.7 nm, -0.005
Max  238.0 nm,  0.008   Min  225.2 nm, -0.010
Max  214.1 nm,  0.014   Min  203.5 nm, -0.115
```

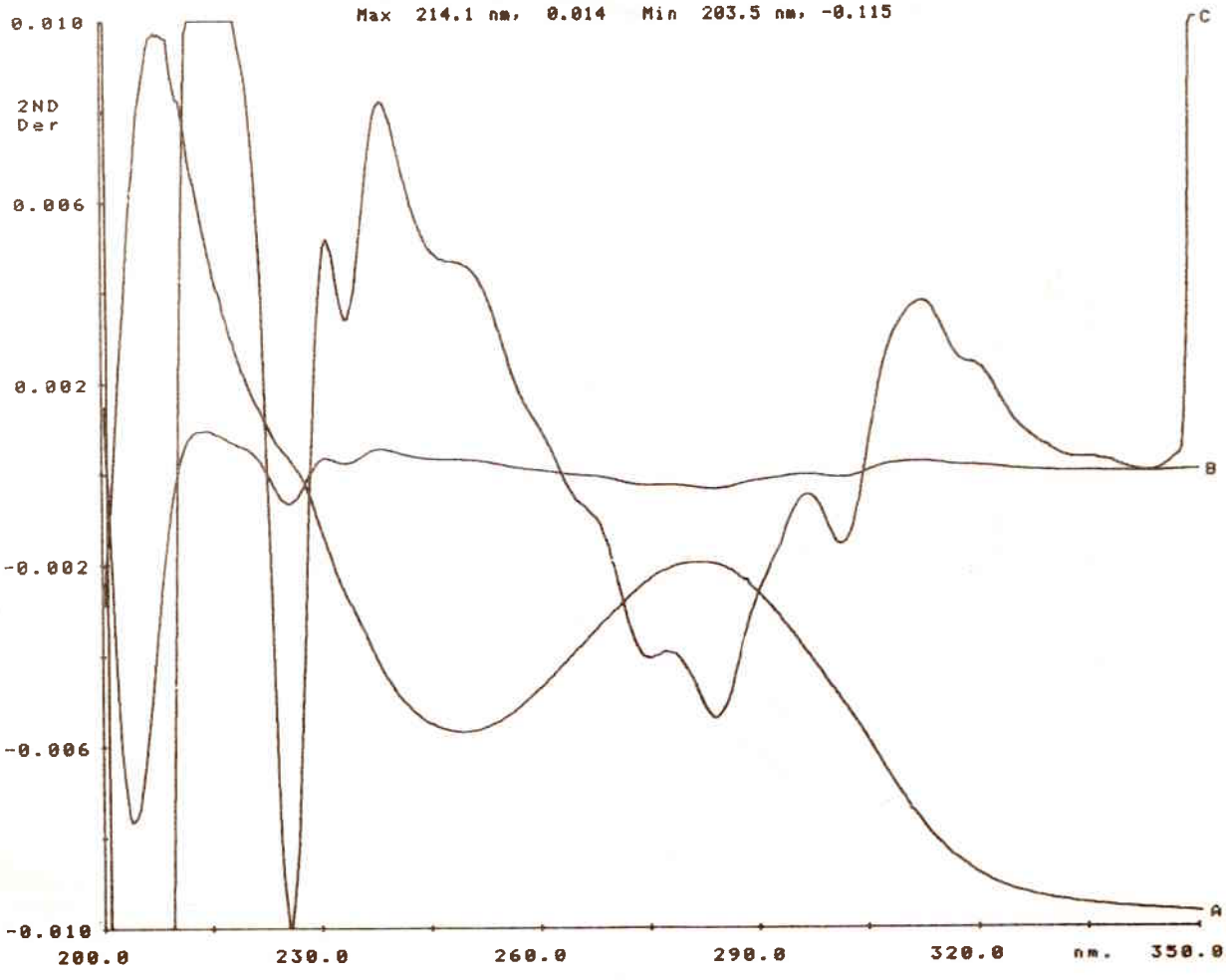

Figure 56

The study of tea infusions with derivative spectroscopy gives certainly more information than the zero order spectra. A 'normal' tea spectrum (Figure 56) shows essentially two peaks only. However, these peaks are shown to have a much more complex structure in higher order derivative spectra (Figure 57).

```
Trace   A                       Operator            Sample
Mode    Abs                     SBW (nm)   1.0      Smoothing (sec)   0
Ord Max/Min    4.000    0.000   WL Max/Min (nm)  450.0   190.0
Speed (nm/min)   100
    35 Peaks, threshold  0.100
Min  449.2 nm,  0.043   Min  363.8 nm,  0.144
Min  317.2 nm,  0.271   Min  291.2 nm,  0.524
Max  272.8 nm,  0.838   Min  249.6 nm,  0.613
Min  214.8 nm,  2.772   Min  213.3 nm,  2.916
Min  212.5 nm,  3.004   Min  211.0 nm,  3.143
Min  210.5 nm,  3.219   Max  209.8 nm,  3.356
Min  209.3 nm,  3.119   Max  208.2 nm,  3.637
Min  207.5 nm,  3.244   Max  207.0 nm,  3.487
Min  206.7 nm,  3.311   Max  205.7 nm,  3.964
Min  205.4 nm,  3.487   Max  205.2 nm,  3.624
Min  204.2 nm,  3.265   Max  203.7 nm,  3.525
Min  203.4 nm,  3.356   Max  203.0 nm,  3.556
Max  202.4 nm,  3.342   Max  201.6 nm,  3.305
Min  201.1 nm,  3.160   Max  200.4 nm,  3.356
Min  200.1 nm,  3.244   Max  199.7 nm,  3.390
Max  198.9 nm,  3.244   Max  198.4 nm,  3.119
Max  196.3 nm,  3.026   Max  195.5 nm,  2.901
Max  191.2 nm,  2.464
```

```
Trace   B                       Operator            Sample
Mode    2ND Der                 SBW (nm)   1.0      Smoothing (sec)
Ord Max/Min    0.010   -0.010   WL Max/Min (nm)  450.0   190.0
Speed (nm/min)   100
    10 Peaks, threshold  0.005
Max  292.8 nm,  0.003   Min  268.9 nm, -0.004
Max  242.7 nm,  0.009   Min  230.5 nm,  0.003
Max  216.6 nm,  0.009   Min  210.3 nm, -0.025
Max  207.5 nm, -0.013   Min  204.2 nm, -0.058
Max  199.8 nm,  0.006   Min  195.6 nm, -0.029
```

```
Trace   C                       Operator            Sample
Mode    2ND Der                 SBW (nm)   1.0      Smoothing (sec)
Ord Max/Min    0.060   -0.060   WL Max/Min (nm)  450.0   190.0
Speed (nm/min)   100
    10 Peaks, threshold  0.005
Max  292.8 nm,  0.003   Min  268.9 nm, -0.004
Max  242.7 nm,  0.009   Min  230.5 nm,  0.003
Max  216.6 nm,  0.009   Min  210.3 nm, -0.025
Max  207.5 nm, -0.013   Min  204.2 nm, -0.058
Max  199.8 nm,  0.006   Min  195.6 nm, -0.029
```

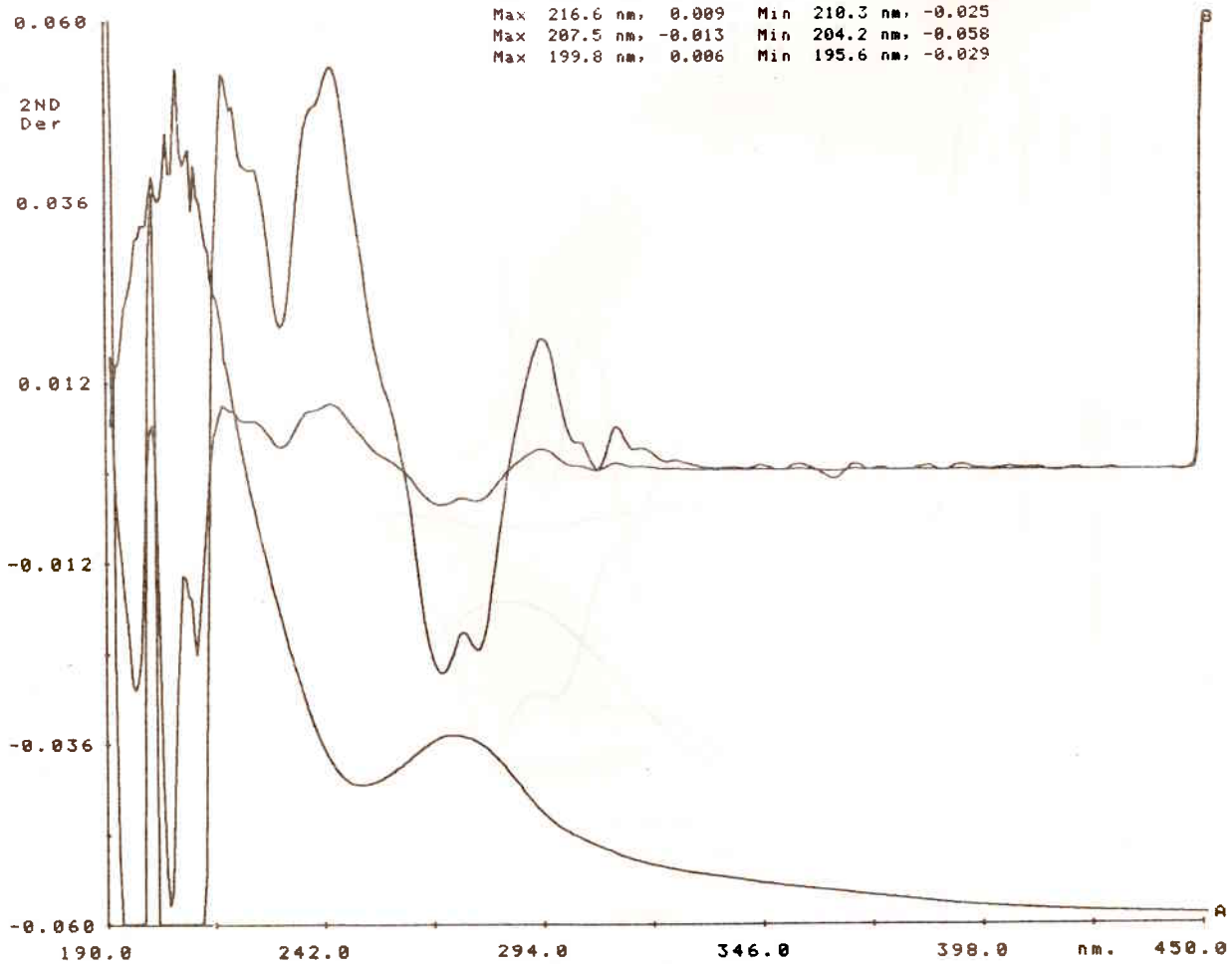

Figure 57

A camomile tea infusion shows very broad peaks which can be resolved into a number of sharp peaks (Figure 58).

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   1.0        Smoothing (sec)   0
Ord Max/Min    3.000    0.000      WL Max/Min (nm)  450.0    200.0
Speed (nm/min)   100
    22 Peaks, threshold  0.100
Min  449.7 nm,  0.042   Min  391.5 nm,  0.147
Min  350.7 nm,  0.457   Min  343.5 nm,  0.574
Min  334.9 nm,  0.678   Min  322.0 nm,  0.781
Min  291.2 nm,  0.888   Min  215.6 nm,  2.161
Min  212.8 nm,  2.401   Min  210.8 nm,  2.571
Min  208.3 nm,  2.772   Max  206.2 nm,  3.026
Min  206.0 nm,  2.921   Max  205.7 nm,  3.079
Min  205.5 nm,  2.964   Min  204.2 nm,  3.055
Min  203.2 nm,  3.191   Max  202.6 nm,  3.311
Min  202.4 nm,  3.089   Max  202.1 nm,  3.305
Min  201.9 nm,  3.082   Max  201.2 nm,  3.398

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   1.0        Smoothing (sec)   1
Ord Max/Min    0.010   -0.010      WL Max/Min (nm)  450.0    200.0
Speed (nm/min)   100
     5 Peaks, threshold  0.005
Min  264.9 nm, -0.001   Min  239.0 nm,  0.001
Max  224.5 nm,  0.008   Max  207.4 nm,  0.000
Min  203.2 nm, -0.019
```

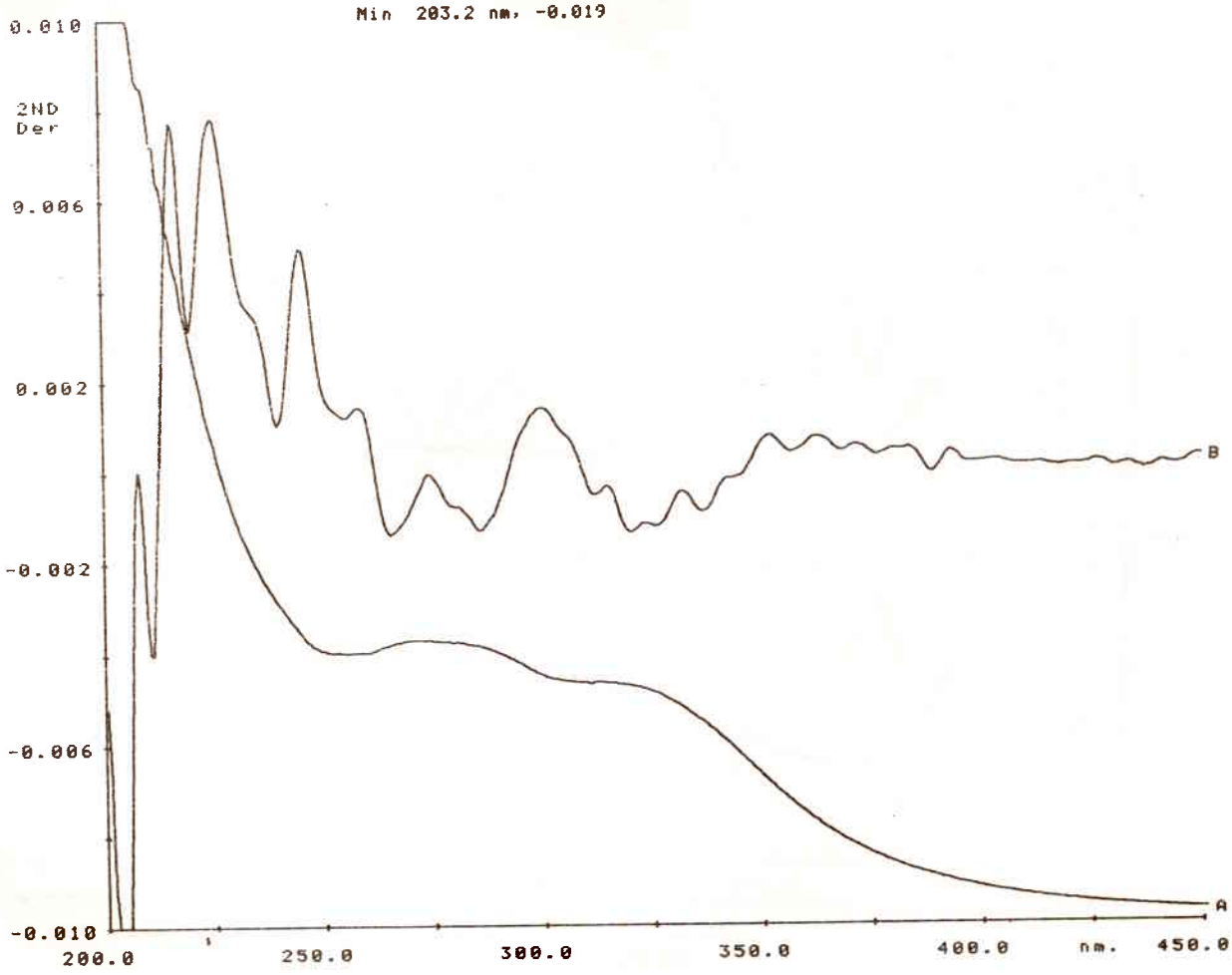

Figure 58

Similar higher order derivative resolution is achieved on a mint tea infusion (Figure 59).

```
Trace   A                          Operator            Sample
Mode    Abs                        SBW (nm)   1.0      Smoothing (sec)   8
Ord Max/Min     4.000    0.000     WL Max/Min (nm)  450.0   190.0
Speed (nm/min)   100
    26 Peaks, threshold  0.100
Min  448.9 nm,  0.034   Min  350.9 nm,  0.395
Min  310.5 nm,  0.526   Max  283.4 nm,  0.717
Min  261.3 nm,  0.611   Min  208.5 nm,  2.530
Min  207.2 nm,  2.715   Min  205.2 nm,  2.834
Min  204.4 nm,  2.867   Min  203.5 nm,  3.022
Min  202.1 nm,  3.089   Max  201.1 nm,  3.209
Min  200.6 nm,  3.086   Max  199.6 nm,  3.224
Min  198.3 nm,  3.089   Max  197.6 nm,  3.249
Min  197.3 nm,  3.147   Max  196.8 nm,  3.293
Min  196.3 nm,  3.089   Max  196.0 nm,  3.234
Min  195.6 nm,  3.115   Max  194.8 nm,  3.229
Min  194.5 nm,  3.022   Max  194.0 nm,  3.156
Max  193.6 nm,  3.026   Max  192.2 nm,  2.890
```

```
Trace   B                          Operator            Sample
Mode    2ND Der                    SBW (nm)   1.0      Smoothing (sec)
Ord Max/Min     0.050  -0.050     WL Max/Min (nm)  450.0   190.0
Speed (nm/min)   100
    10 Peaks, threshold  0.005
Max  295.8 nm,  0.002   Min  284.2 nm, -0.004
Min  250.9 nm,  0.001   Max  236.1 nm,  0.007
Min  228.6 nm,  0.000   Max  213.6 nm,  0.011
Max  207.4 nm,  0.005   Min  201.6 nm, -0.036
Max  197.8 nm, -0.007   Min  193.5 nm, -0.053
```

```
Trace   C                          Operator            Sample
Mode    2ND Der                    SBW (nm)   1.0      Smoothing (sec)
Ord Max/Min     0.005  -0.005     WL Max/Min (nm)  450.0   190.0
Speed (nm/min)   100
    10 Peaks, threshold  0.005
Max  295.8 nm,  0.002   Min  284.2 nm, -0.004
Min  250.9 nm,  0.001   Max  236.1 nm,  0.007
Min  228.6 nm,  0.000   Max  213.6 nm,  0.011
Max  207.4 nm,  0.005   Min  201.6 nm, -0.036
Max  197.8 nm, -0.007   Min  193.5 nm, -0.053
```

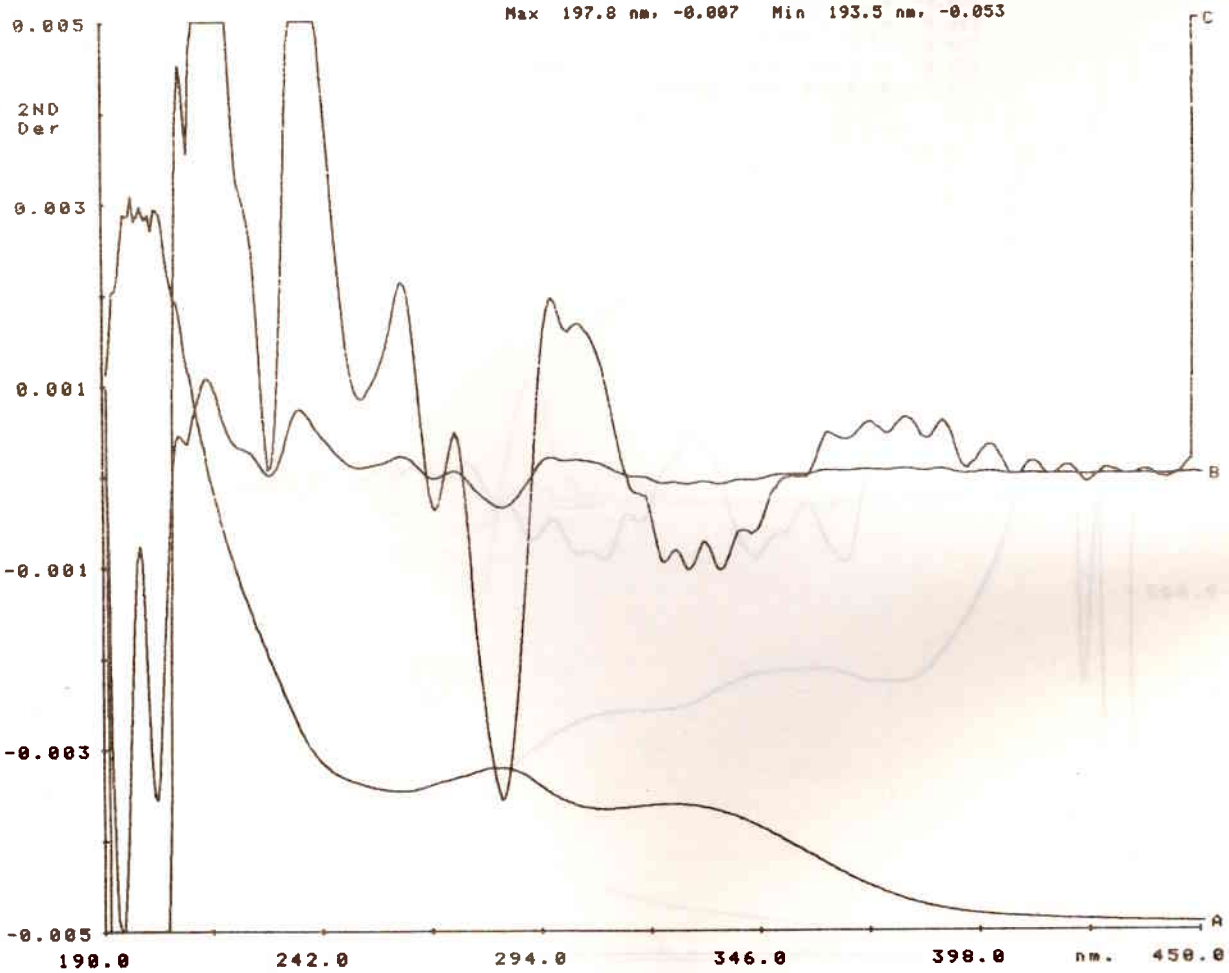

Figure 59

Transparent, solid materials, such as plastic films, also often exhibit rather broad absorption peaks in their zero order spectra, but these can be readily resolved with higher order derivative spectroscopy (Figure 60).

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   1.0        Smoothing (sec)   0
Ord Max/Min     1.500  -0.005      WL Max/Min (nm)  700.0   320.0
Speed (nm/min)   200               B'line Corr
     7 Peaks, threshold  0.100
Min  697.5 nm,  0.080   Min  617.9 nm,  0.172
Min  550.9 nm,  0.928   Max  510.7 nm,  1.415
Min  401.8 nm,  0.582   Min  350.6 nm,  1.131
Min  335.5 nm,  1.285

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   1.0        Smoothing (sec)   1
Ord Max/Min     0.010  -0.010      WL Max/Min (nm)  700.0   320.0
Speed (nm/min)   200               B'line Corr
     9 Peaks, threshold  0.005
Max  588.5 nm,  0.003   Min  559.5 nm, -0.003
Max  535.4 nm,  0.002   Min  512.9 nm, -0.004
Min  476.0 nm, -0.003   Min  383.2 nm, -0.000
Max  361.8 nm,  0.008   Min  349.2 nm, -0.008
Max  325.2 nm,  0.022
```

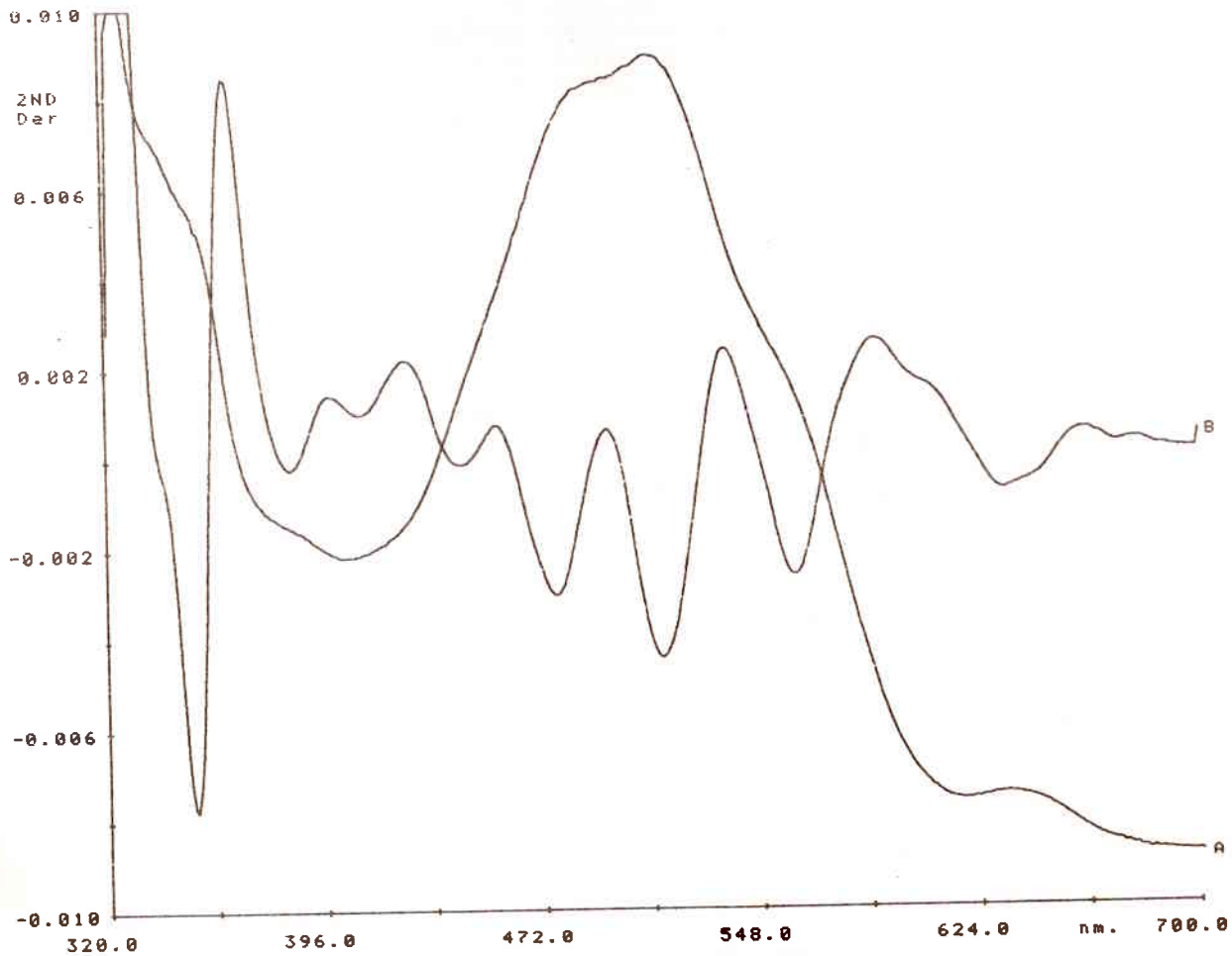

Figure 60

Alcoholic beverages often exhibit rather featureless spectra which can however be considerably enhanced by the use of derivative techniques.

An intensly colorful liqueur, such as BLUE CURACAO, exhibits three peaks in the UV-Visible region (Figure 61) each of which can be readily resolved into several peaks in the derivative spectrum.

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)    1.0       Smoothing (sec)   0
Ord Max/Min     1.000    0.000     WL Max/Min (nm)  700.0   190.0
Speed (nm/min)   100
    17 Peaks, threshold  0.050
Min  638.1 nm,  0.021   Min  671.0 nm,  0.155
Max  639.0 nm,  0.787   Max  589.3 nm,  0.270
Max  554.7 nm,  0.091   Min  467.7 nm,  0.017
Max  410.8 nm,  0.109   Min  350.7 nm,  0.050
Min  283.1 nm,  0.149   Min  247.6 nm,  0.203
Min  233.4 nm,  0.327   Max  196.1 nm,  2.641
Min  195.5 nm,  2.572   Max  195.0 nm,  2.714
Min  194.5 nm,  2.572   Max  194.3 nm,  2.674
Max  192.0 nm,  2.404

Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)    1.0       Smoothing (sec)   1
Ord Max/Min     0.004   -0.006     WL Max/Min (nm)  700.0   190.0
Speed (nm/min)   100
     7 Peaks, threshold  0.005
Max  664.3 nm,  0.002   Min  640.0 nm, -0.005
Min  311.0 nm, -0.002   Max  213.8 nm,  0.012
Min  207.0 nm,  0.002   Max  199.3 nm,  0.035
Min  192.9 nm, -0.106
```

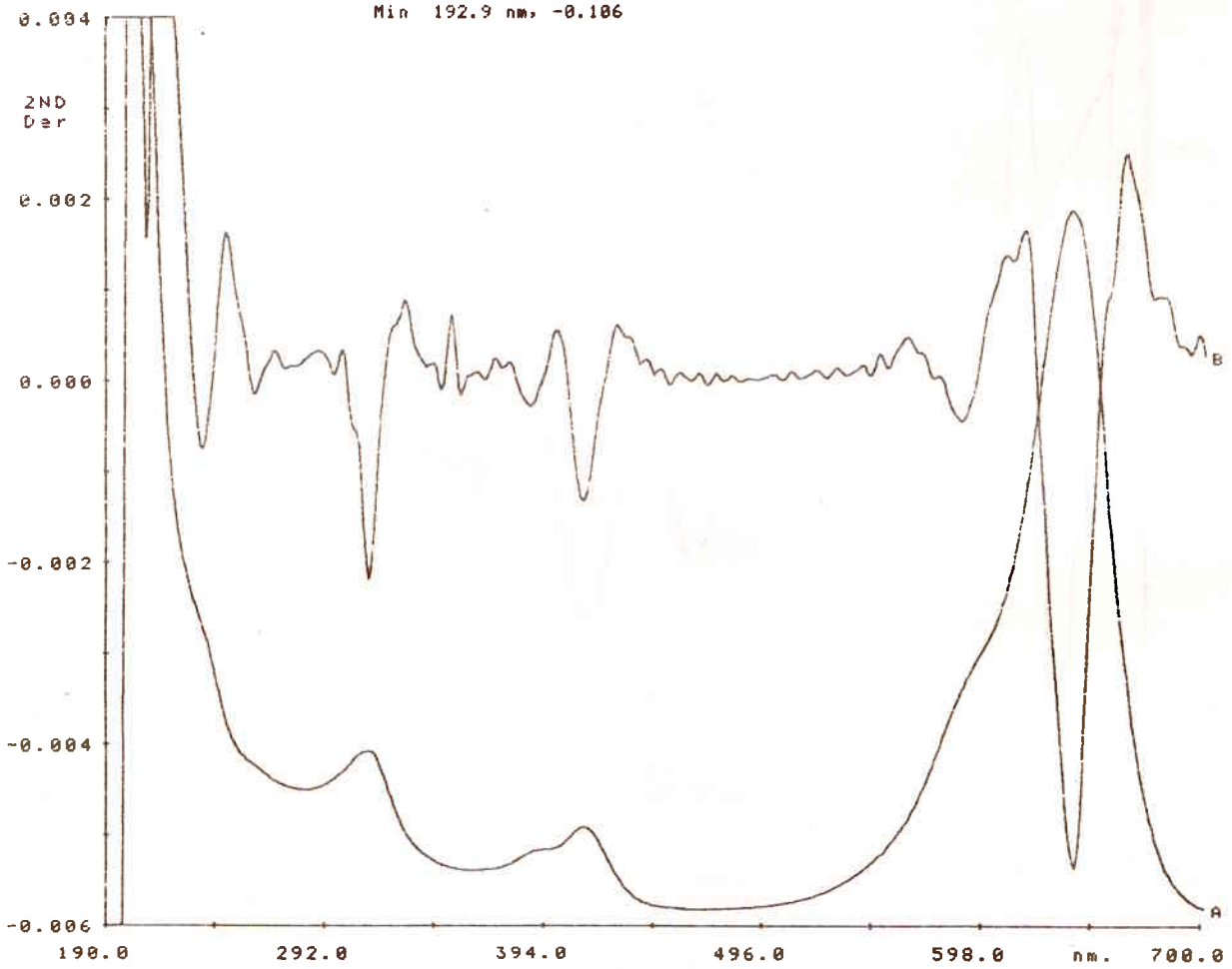

Figure 61

Similarly, the digestive liquers FERNET BRANCA and MENTA show virtually identical, poorly resolved zero order spectra which are resolved into several quite distinctive peaks with derivative spectroscopy (Figure 62 and Figure 63).

```
Trace   A                          Operator              Sample
Mode    Abs                        SBW (nm)   1.0        Smoothing (sec)   0
Ord Max/Min    3.000    0.000      WL Max/Min (nm)  390.0    190.0
Speed (nm/min)   100
    20 Peaks, threshold  0.050
Min  386.9 nm,  0.194   Min  367.6 nm,  0.248
Min  350.9 nm,  0.305   Min  314.5 nm,  0.860
Min  310.4 nm,  0.920   Max  299.0 nm,  1.043
Max  285.9 nm,  0.944   Max  280.8 nm,  0.888
Min  263.7 nm,  0.690   Min  249.5 nm,  1.050
Min  244.2 nm,  1.104   Min  223.2 nm,  1.674
Min  221.1 nm,  1.724   Min  218.6 nm,  1.791
Min  216.5 nm,  1.846   Min  203.9 nm,  1.873
Min  198.7 nm,  1.943   Min  194.5 nm,  2.069
Min  192.5 nm,  2.155   Max  191.2 nm,  2.260
```

```
Trace   B                          Operator              Sample
Mode    2ND Der                    SBW (nm)   1.0        Smoothing (sec)   1
Ord Max/Min    0.025   -0.025      WL Max/Min (nm)  390.0    190.0
Speed (nm/min)   100
    11 Peaks, threshold  0.005
Max  388.7 nm,  0.001   Min  304.8 nm, -0.004
Max  258.2 nm,  0.020   Min  252.1 nm, -0.024
Max  246.4 nm,  0.005   Min  243.2 nm,  0.000
Max  238.4 nm,  0.008   Min  224.7 nm, -0.004
Max  218.0 nm,  0.001   Min  211.5 nm, -0.008
Max  196.9 nm,  0.010
```

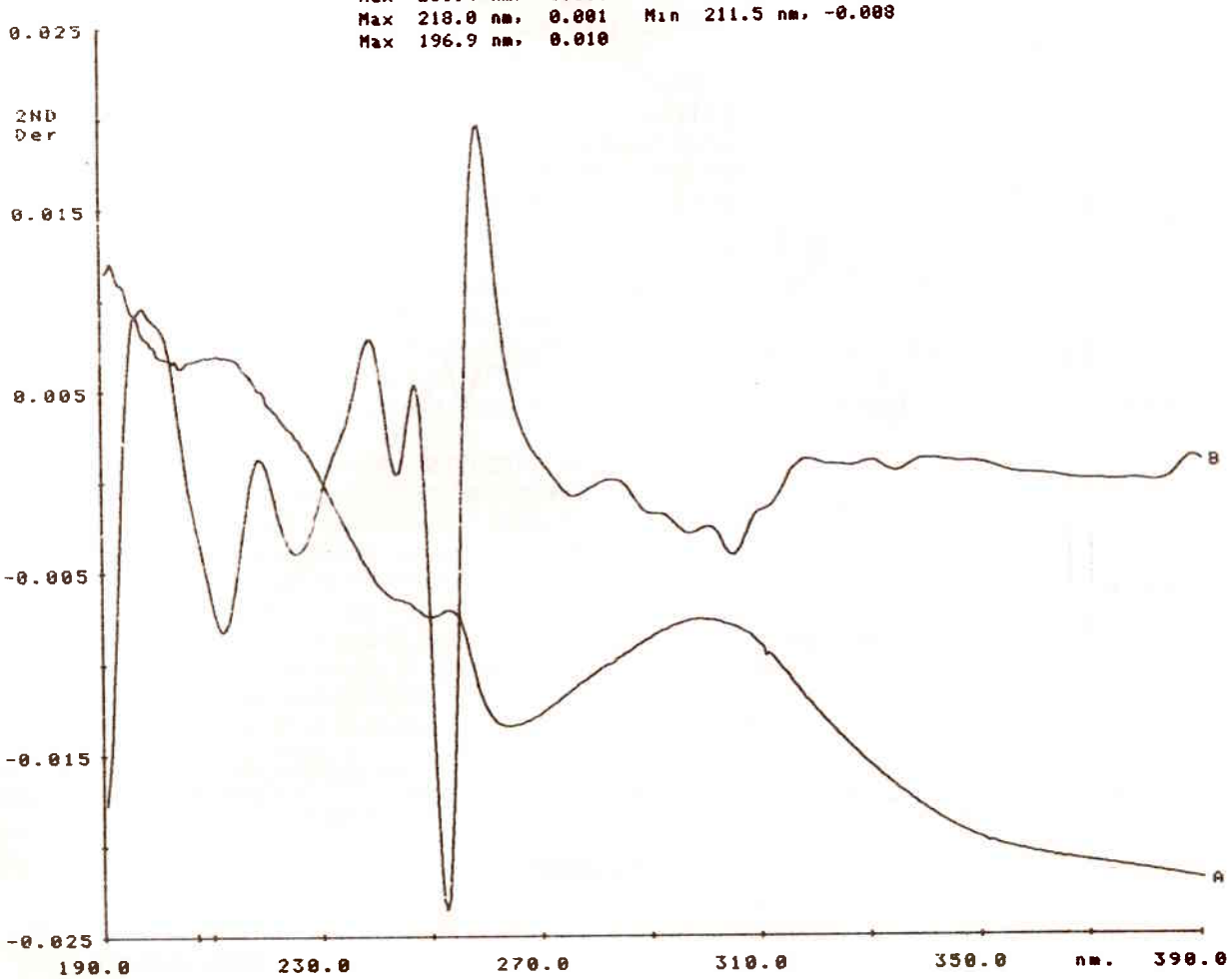

Figure 62

```
Trace   A                       Operator            Sample
Mode    Abs                     SBW (nm)   1.0      Smoothing (sec)   0
Ord Max/Min    3.000   0.000    WL Max/Min (nm)  390.0   190.0
Speed (nm/min)   100
    40 Peaks, threshold  0.050
Min  388.4 nm,  0.211   Min  372.2 nm,  0.264
Min  359.0 nm,  0.323   Min  350.8 nm,  0.374
Min  310.4 nm,  1.233   Min  306.7 nm,  1.339
Max  299.8 nm,  1.392   Max  290.6 nm,  1.330
Max  285.8 nm,  1.261   Min  266.0 nm,  0.974
Min  250.0 nm,  1.586   Min  245.8 nm,  1.576
Min  237.3 nm,  1.752   Min  228.5 nm,  2.127
Min  225.9 nm,  2.207   Min  222.9 nm,  2.273
Min  220.8 nm,  2.347   Min  219.3 nm,  2.383
Min  217.8 nm,  2.443   Min  216.8 nm,  2.483
Max  213.7 nm,  2.573   Min  211.2 nm,  2.506
Max  210.9 nm,  2.573   Max  209.5 nm,  2.554
Min  203.3 nm,  2.464   Min  201.1 nm,  2.521
Min  199.3 nm,  2.572   Min  198.0 nm,  2.667
Min  197.3 nm,  2.704   Min  196.3 nm,  2.765
Min  195.4 nm,  2.797   Max  194.0 nm,  3.009
Min  193.9 nm,  2.875   Max  193.5 nm,  3.009
Min  193.4 nm,  2.954   Max  193.0 nm,  3.082
Min  192.5 nm,  2.930   Max  192.2 nm,  3.018
Min  191.9 nm,  2.962   Max  191.4 nm,  3.144
```

```
Trace   B                       Operator            Sample
Mode    2ND Der                 SBW (nm)   1.0      Smoothing (sec)
Ord Max/Min    0.030  -0.030    WL Max/Min (nm)  390.0   190.0
Speed (nm/min)   100
    13 Peaks, threshold  0.005
Max  348.5 nm,  0.002   Min  304.6 nm, -0.006
Min  279.2 nm, -0.001   Max  258.4 nm,  0.023
Min  252.3 nm, -0.029   Max  246.5 nm,  0.005
Min  243.4 nm, -0.000   Max  238.4 nm,  0.008
Min  225.4 nm, -0.008   Max  220.4 nm,  0.002
Min  209.7 nm, -0.010   Max  199.8 nm,  0.018
Min  190.7 nm, -0.066
```

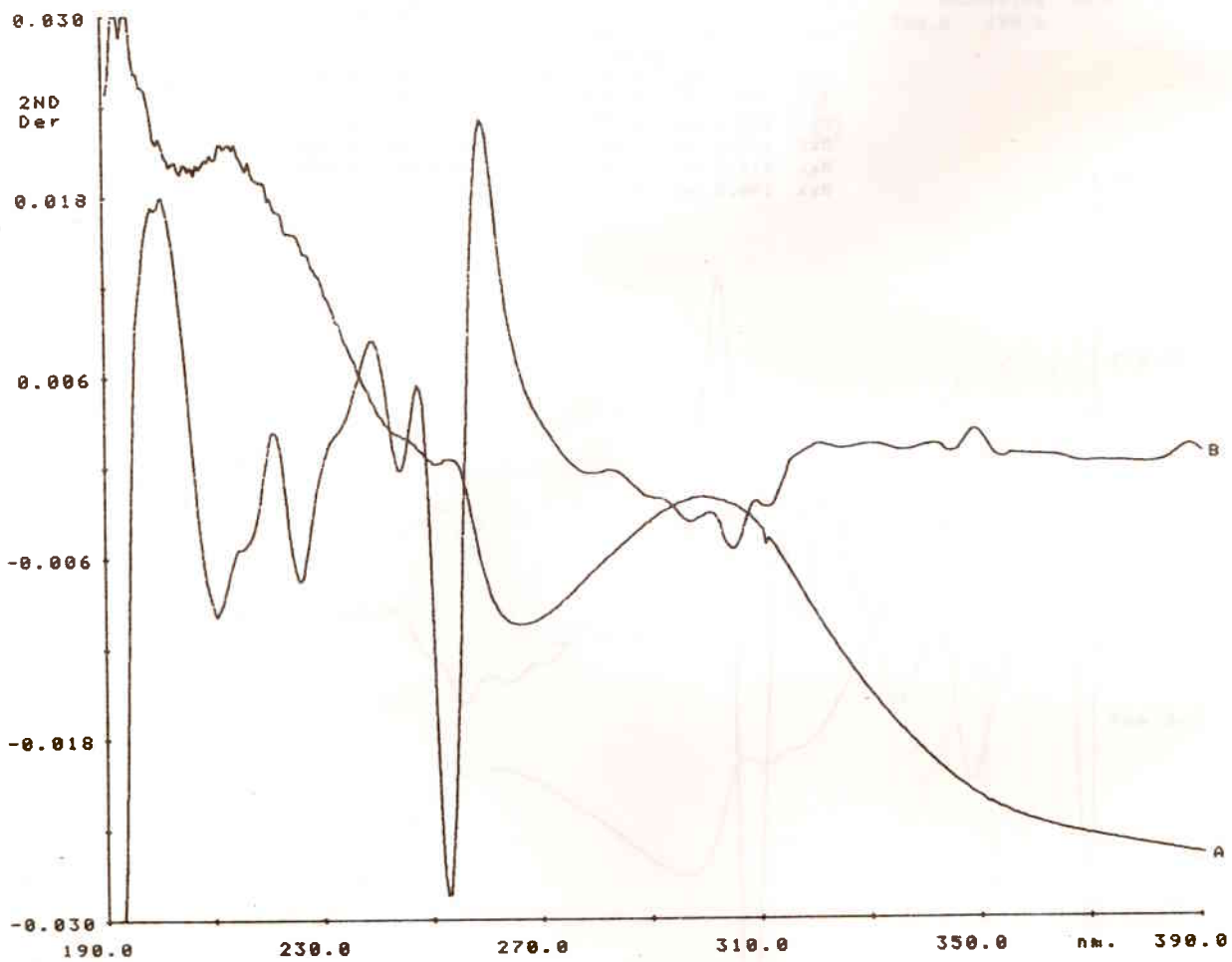

Figure 63

These examples illustrate the huge potential which exists for the application of the derivative technique to the characterization of both natural substances as well as commercial substances.

Conclusion

It is hoped that in this discussion and from the number of illustrative examples presented the usefulness of derivative UV-Visible spectrophotometric techniques for a wide variety of areas of application has been highlighted.

A final word of caution must be introduced, namely that derivative spectra cannot provide any extra information that is not already present in the original zero order absorbance spectra. Derivative spectroscopy simply presents the information in other, visually more easily interpretable format. Thus, it cannot be stressed too highly that the quality and usefulness of derivative spectra will be completely dependent on the intrinsic performance of the UV-Visible spectrophotometer used, even when operated under the optimum measurement conditions.

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