The Use of HPLC with Diode Array Detection for the Analysis and Relative Dating of Inks and the Differentiation of Fiber Dyes.

Final Report on grant # 89-IJ-CX-0057

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SUMMARY

High Performance Liquid Chromatographic (HPLC) systems were developed for the examination of non ball pen inks and fiber dyes. Using a simple chromatographic system, together with a Spectra Physics multiwavelength detection system, over 100 non ball pen inks were distinguished. The majority of these had not previously been separated by traditional methods. The system was also found to be useful for the comparison of light colored fiber dyes, where thin layer chromatography and microspectrophotometry had failed to produce useful data. This approach offers a number of advantages over less sophisticated means of dye comparison: Sensitivity is greatly improved, allowing the detection of picograms of sample, and the increased resolution results in far more information being produced, enabling a more meaningful comparison of samples.

The use of HPLC with diode array detection for the analysis and relative dating of inks and the differentiation of fiber dyes.

OBJECTIVE

The forensic examination of writing inks attempts to accomplish two purposes: to determine whether two or more entries were made with the same pen or same formula of ink and/ or to determine the time frame in which an entry was made. The first problem is usually solved through a comparison of the physical and chemical properties of the inks. The second problem is approached by comparing the properties of a questioned sample against a library of samples of known age and origin.

The primary means of chemical analysis is through thin layer chromatographic separation of the non-volatile dye components of the ink. Although many ink formulations differ in their dye constituents, some do not and are difficult to distinguish. This situation presently exists with a family consisting of over a hundred non ball pen inks which cannot be distinguished by present methods. This research was designed to evaluate HPLC with diode array detection for the differentiation of closely related writing inks. It was anticipated that the improved resolution and sensitivity of HPLC compared with TLC would enable minor differences to be detected in these samples. At the same time, a multiwavelength detector such as a diode array would allow the simultaneous detection of many different components of the ink.

This same technique is applicable to the differentiation of fiber dyes, many of these dyes being similar in composition to inks. Present methods of analysis for fiber dyes routinely involve the use of thin layer chromatography, for the separation of the dyes, and microspectrophotometry. The latter allowing the visible spectrum of the dye to be determined. HPLC combines both of these techniques, separating the dye components and allowing their examination in both the visible and ultraviolet regions of the spectrum. HPLC for the differentiation of fiber dyes is still very much in its infancy. This project was designed to investigate the degree to which this technique could be used to improve the differentiation of selected fiber dyes as compared to TLC and microspectrophotometry.

METHODS

Instrumentation

A Perkin Elmer Series 3B liquid chromatographic pump was used to deliver mobile phase to the analytical column. Samples were injected onto the system via a Rheodyne injection valve incorporating a 20ul loop. Detection of the eluting components was achieved with a Spectra Physics Focus detection system and integration and data manipulation was with an IBM Personal System/2 Model 70 computer.

Sample preparation

Inks

Samples of different inks in the form of 1cm x 0.5cm pieces of paper soaked in the ink were obtained from the ink library maintained by the Internal Revenue Service Forensic laboratory, Chicago. A sample consisting of 5mm x 1mm was cut from this reference material and placed in a tapered tube with 50ul of HPLC mobile phase. The suspension was agitated on a sonic bath for 20 minutes to extract the dye, and 20ul of the supernatant was then injected directly onto the HPLC column. The effluent from the column was monitored between 200 and 800 nm in order to detect those components that absorbed in either the visible of UV range. Each sample was extracted 5 times in order to ascertain the reproducibility of the technique. Samples of the same ink but of different batch numbers were also examined as were extracts from different types of paper, to ensure that chemicals present in the paper did not interfere with the analysis.

HPLC

The following HPLC systems were evaluated for their ability to differentiate 17 non ball pen inks representing examples of each of the subsections of the inks under investigation:

1. Column : Spherisorb 5um ODS

Eluent : Acetonitrile : Water (80:20) with 0.005M heptanesulfonic acid and 0.02% acetic acid.

2. Column : Spherisorb 5um
   Eluent : Dichloroethane : Ethanol : Formamide (89:10:1).

3. Column : Spherisorb 5um ODS
   Eluent : Acetonitrile : Tetrahydrofuran : Water (924:432:644) with citric acid (1.75g/L) and hexane sulfonic acid (0.75g/L).

4. Column : Spherisorb 5um ODS
   Eluent : Methanol : Water (60:40) with 0.005M tetra-n-butylammonium phosphate at pH 7.2.

5. Column : Spherisorb 5um
   Eluent : Methanol : Ammonium acetate solution (pH 9.7) (9:1).

In each case, the flow rate was maintained at between 1-2 ml/min.

Fibers

Examples of each of the major classes of fiber dyes, namely; acid, acid mordent, direct, reactive, solubilized VAT, sulfur, leucosulfur, basic, mordant, disperse and reactive, were extracted by the following procedure. Single fibers, typically 0.5 to 1cm in length were placed in a capillary tube sealed at on end, together with approximately 100ul of pyridine:water (4:3) or formic acid:water (1:1). The open end of the tube was then heat sealed

and the tube placed in a sand bath at 100 C for 20 minutes. Once the dye had been extracted from the fiber, the top of the capillary tube was snapped off and the solvent transferred to a tapered glass tube. The solvent was evaporated to dryness under a stream of nitrogen and the residue reconstituted in 100ul of methanol. These extracts were subjected to thin layer chromatographic analysis as well as examination by HPLC. The visible absorbance spectra of the dyes was also obtained by placing single fibers mounted in Permamount, under a Nanospec microspectrophotometer, and scanning over the range 400-800nm.

TLC

The following TLC systems were used for the separation of the various dye types:

Direct dyes : n-Butanol, ethanol, conc. Ammonia, pyridine, water. (8:3:4:4:3).

Reactive dyes : Methanol, amyl alcohol, water (5:5:2).

Disperse dyes : Toluene, pyridine (4:1).

Acid dyes : Chloroform, water, methanol, ammonia (11:1:7:1).

Metal-Complex dyes : n-Butanol, pyridine, water (2:2:1).

Basic dyes : Chloroform, isopropanol, pyridine, water (6:8:3:1:1).

In each case, chromatography was performed on 0.25mm silica gel G plates (Merck).

Each chromatogram was examined under normal daylight and ultraviolet light. Where applicable, densitometry was performed on the TLC plates as a measure of the separation and resolution of the dye components.

HPLC

HPLC was performed on the fiber dyes by injecting 20ul of the methanolic extract onto the chromatographic column. Each of the HPLC systems previously described for the examination of inks were evaluated for their ability to separate these dye components.

RESULTS & DISCUSSION

Inks

Of the five liquid chromatographic methods evaluated for their ability to distinguish and identify each of 17 groups of blue non ball pen inks, (labelled A through O), the method found to give optimal separation consisted of a Spherisorb 5um ODS column with a mobile phase of acetonitrile:water (80:20) with 0.005M heptane sulphonic acid at a pH of 4.7. The flow rate was 1ml/min and the eluent was monitored at all wavelengths in the UV and visible regions (200-800nm). Each ink sample was extracted as previously described with 50ul of mobile phase, and 20ul was injected onto the HPLC.

Each of the 17 groups of ink were readily separated by this method and representative chromatograms are enclosed Figs 1-5.
Reproducibility of the technique was determined by repeated extraction and analysis of each sample (5 times). All samples in our collection were then examined by this method, amounting to over 100 different inks. Only group A, the largest group, consisting of

32 samples, produced some chromatograms which could not be immediately distinguished from other inks in the group. With this group it was necessary to examine the ultraviolet and visible spectra of each eluting peak in the chromatogram, in order to determine differences in the composition of the inks. Where necessary, the absorbance ratios ie. the ratio of spectral absorbance at one wavelength to the absorbance at a second, third and fourth wavelength, were calculated to further aid in the differentiation of individual inks. Figs 6,7 & 8. Using this approach, all inks in the collection could be distinguished.

While we initially expected to be able to separate the inks based on their absorbance characteristics in the visible spectrum, this was not found to be the case. The chromatograms in the visible range being virtually identical for most of the inks examined. However major differences were seen in the chromatograms in the ultraviolet region. In retrospect this is not surprising, as all the samples examined were blue inks, it is likely that the manufacturers employ similar dyestuffs, but with different vehicles (the non colored fraction) which absorb in the UV region of the spectrum.

The great advantage that this type of detection system has over traditional HPLC detectors is that complete chromatographic and spectral data can be collected simultaneously using a sample size of a few nanograms. Those inks which cannot be immediately differentiated based on their chromatographic data can be further

examined by comparison of their UV or visible spectra and, if required, the derivative spectra of individual peaks.

We have shown that by using a simple isocratic HPLC system, together with the highly sensitive Spectra Physics detection system, even very closely related inks can be distinguished. In this study a 5mm line cut from the document was used for the analysis. However, the sensitivity of the technique suggests that meaningful data could be obtained from a much smaller sample size if required. Differences in the data obtained from different batches of the same ink were minor and did not interfere with the identification and comparison of the inks.

Fiber Dyes

All samples of fiber dyes in our collection were examined by microspectrophotometry (nanospec). This technique appears to offer a rapid and non destructive method for the comparison of all types of fiber and all dye colors, with the exception of very light colors, such as yellow and orange, and very dark colors, such as black and dark blue. With light colored dyes, the visible spectrum is too weak to be useful for the comparison of similar dyestuffs. Conversely, dark colored dyes absorb so much of the incident light that the spectra obtained show little variation between different fibers.

Those fibers which could not be distinguished by this technique were extracted with a suitable solvent and further examined by thin

layer chromatography. TLC allowed the separation of the dark fiber dyes, which were generally found to consist of several dyestuffs. However light colored fiber dyes usually only produced one weak spot on the thin layer chromatographic plate, making a comparison of these dyes difficult. Densitometry of these chromatograms produced only single diffuse peaks (Fig 9). These light colored fiber dyes were therefore selected for further examination by HPLC.

Both reversed phase and normal silica HPLC systems were evaluated for their ability to separate a range of different types of light colored dyes. The diverse nature of these dyes has necessitated a normal silica column to be used for their separation, together with a mobile phase consisting of dichloroethane : ethanol : formamide (89:9:1). Coupled with the multiwavelength detection system, this method enabled the differentiation of all fiber dyes examined (Figs 10 & 11).

As with the examination of inks, the use of HPLC with the multiwavelength detector offers several advantages over traditional methods of analysis for fiber dyes. The resolution of dye components by this technique is far superior to that possible by TLC. Where TLC produced a single diffuse spot for pale yellow fibers, HPLC resulted in the separation of several components making a comparison of different fibers more meaningful. The sensitivity of the technique is also attractive to forensic applications. Even with single fibers, the instrument was operated at the lower end of its sensitivity setting, making this approach

applicable to the smallest of samples. The combination of HPLC with a multiwavelength detector essentially gives the same information as both TLC and nanospec combined, since both chromatographic and spectral data can be obtained simultaneously.

CONCLUSION

The increased sensitivity offered by this technique, enables meaningful data to be obtained from samples previously considered to be too small to work with. In addition the improved resolution associated with HPLC separations, together with the data handling capabilities of this instrument, allow even closley related samples to be differentiated. This in turn gives the examiner a greater degree of certainty concerning the comparison of two samples, and may result in greater evidential value being placed on this type of analysis.

This work will be presented at the International Association of Forensic Sciences, Adelaide, Australia (October 1990) and at the American Academy of Forensic Sciences meeting Anaheim, Ca (February 1991). The technical data will be submitted for publication by the Journal of Forensic Sciences.

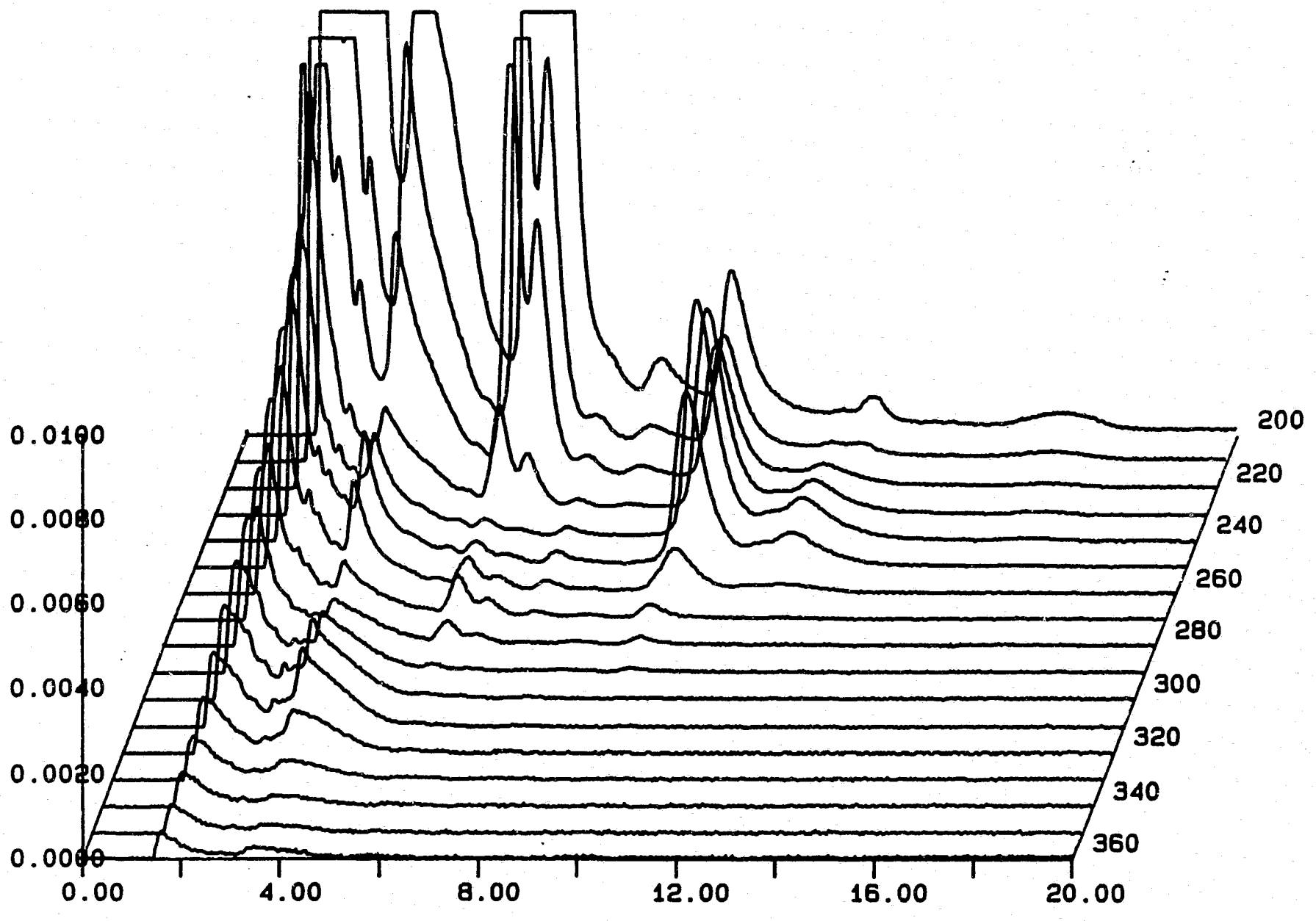
Figures

1-5 Representative HPLC chromatograms of non ball pen inks with multiwavelength detection.

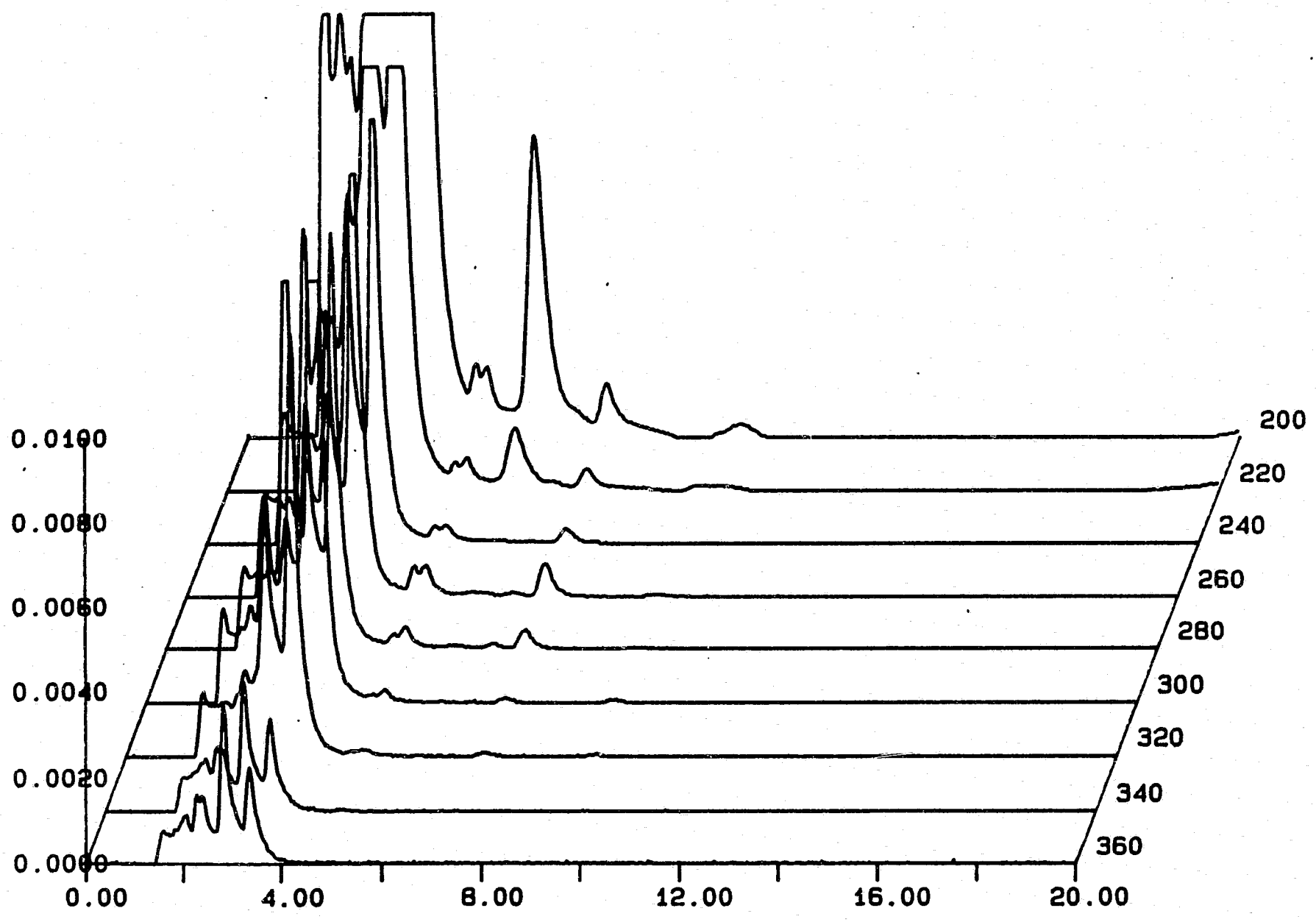
6,7,8 Distinction of closely related inks (#'s 8,11 &22, Group A) by examination of the ultraviolet spectra of the chromatographic peak at 1.48 minutes.

9. Densitometry of a TLC chromatogram of a yellow disperse dye from a single polyester fiber. This represents one diffuse spot on the chromatogram.

10,11. HPLC analysis of the same dye extract as in Figure 9. Note the vast increase in information obtained by the HPLC method as compared with TLC.



Chromatogram Display: \INKHL____.BFF



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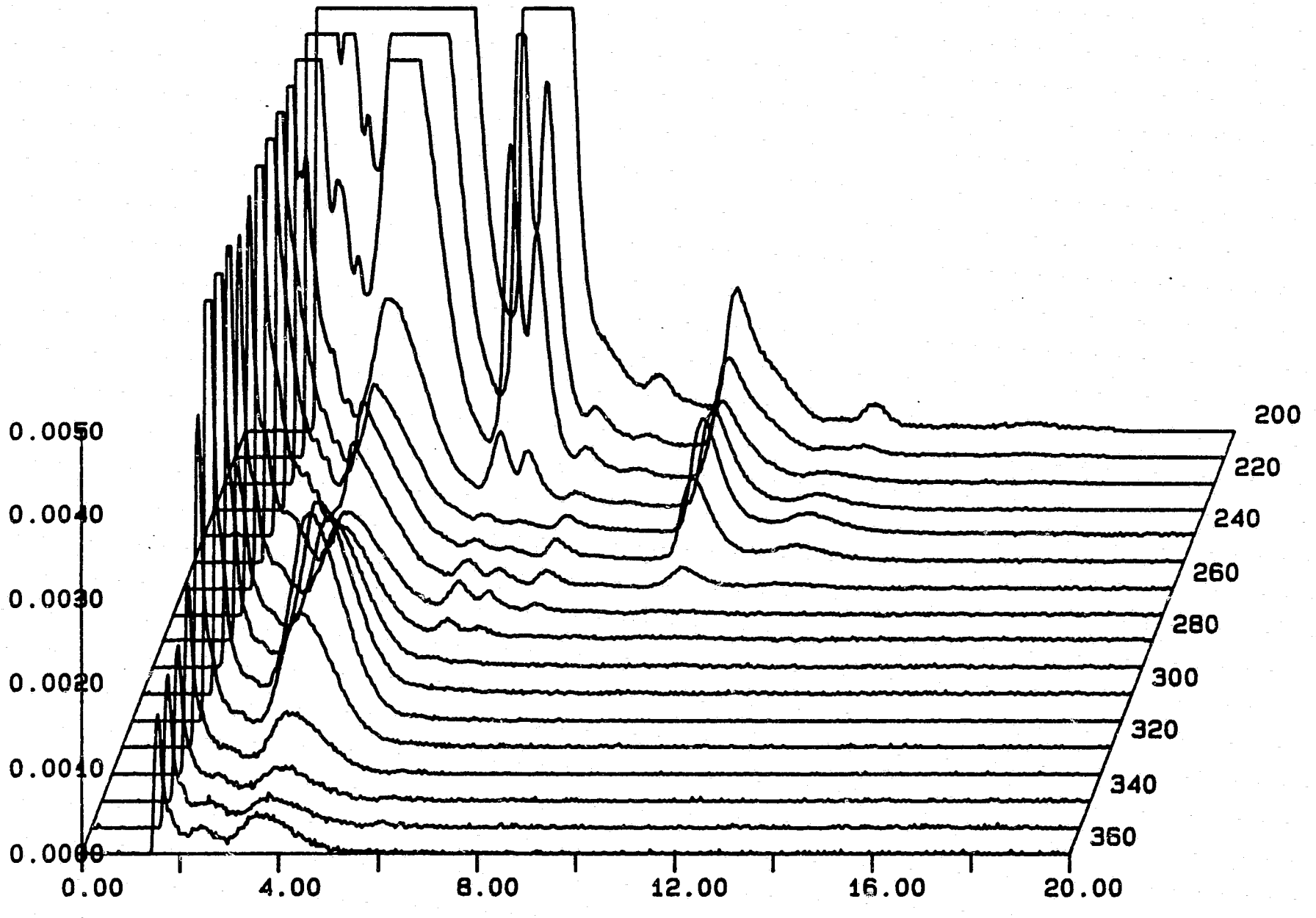

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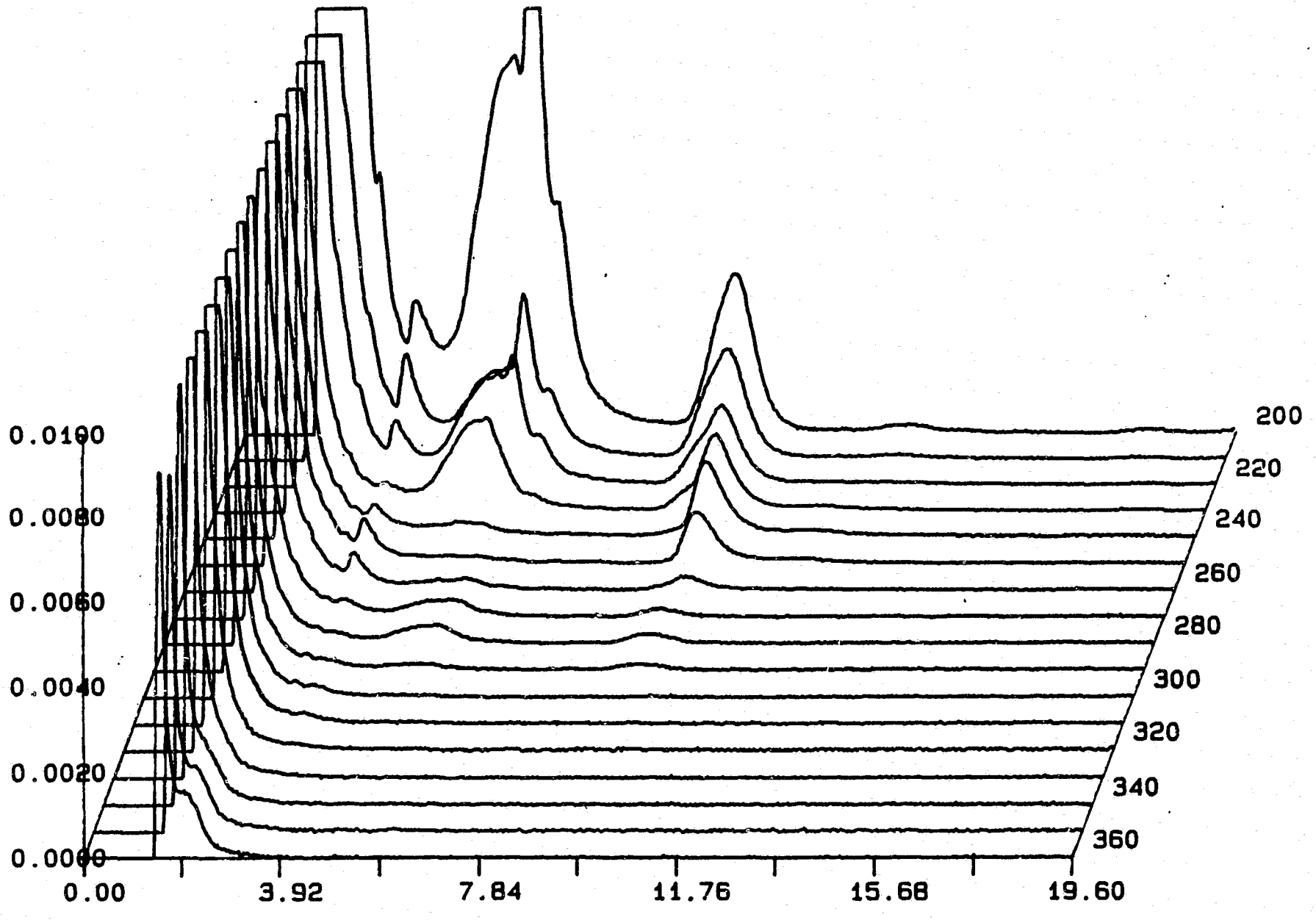

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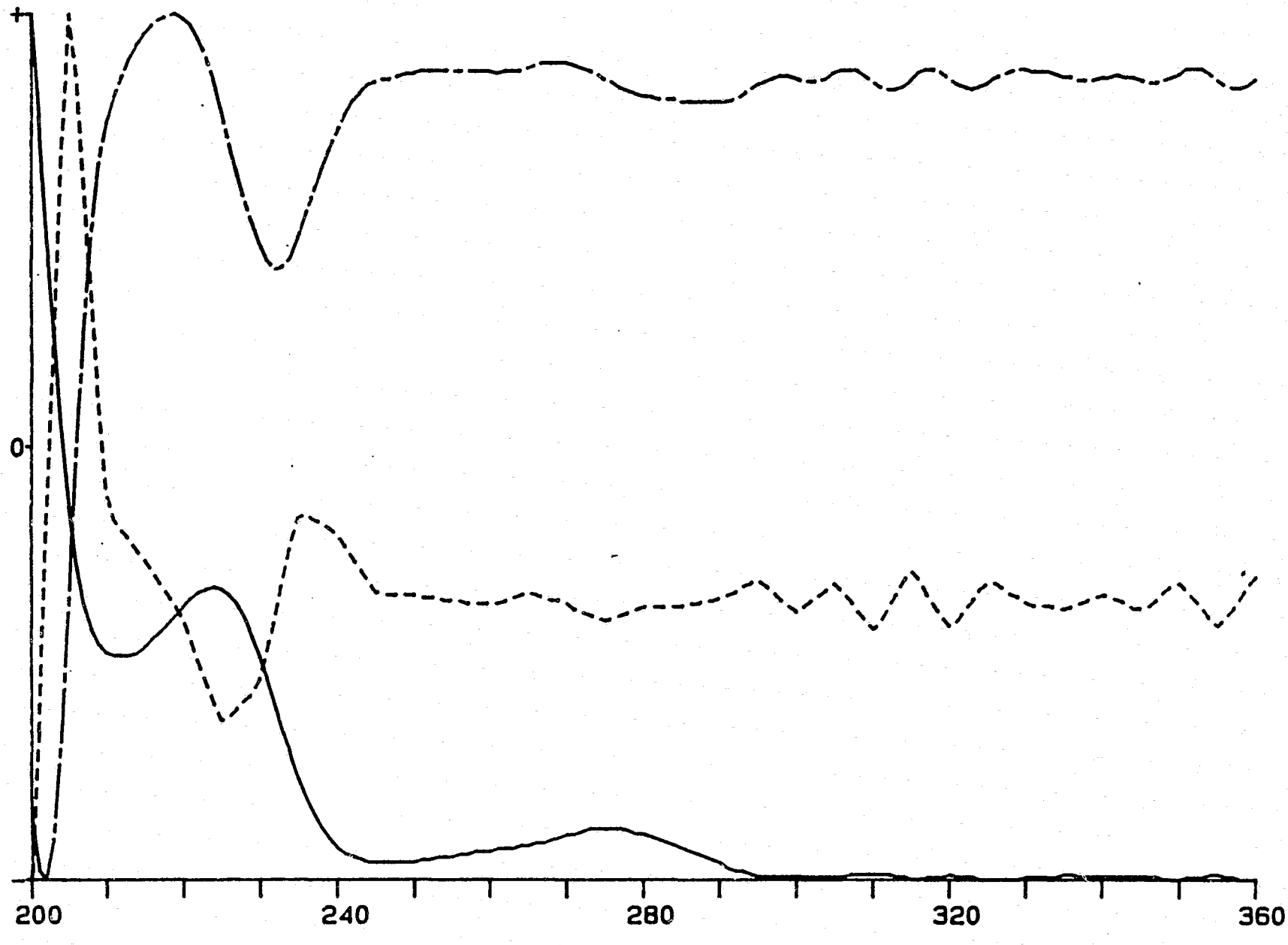

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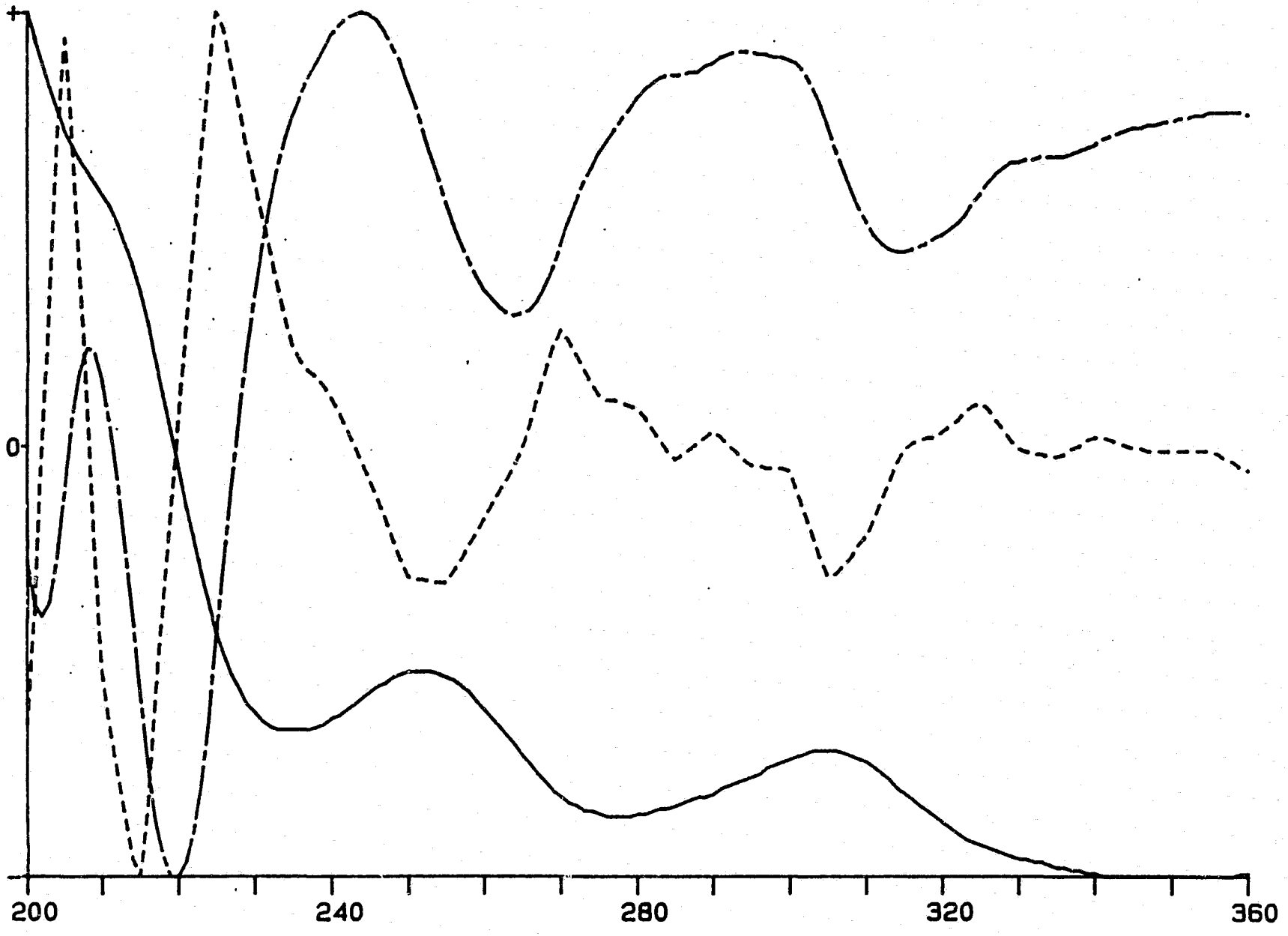

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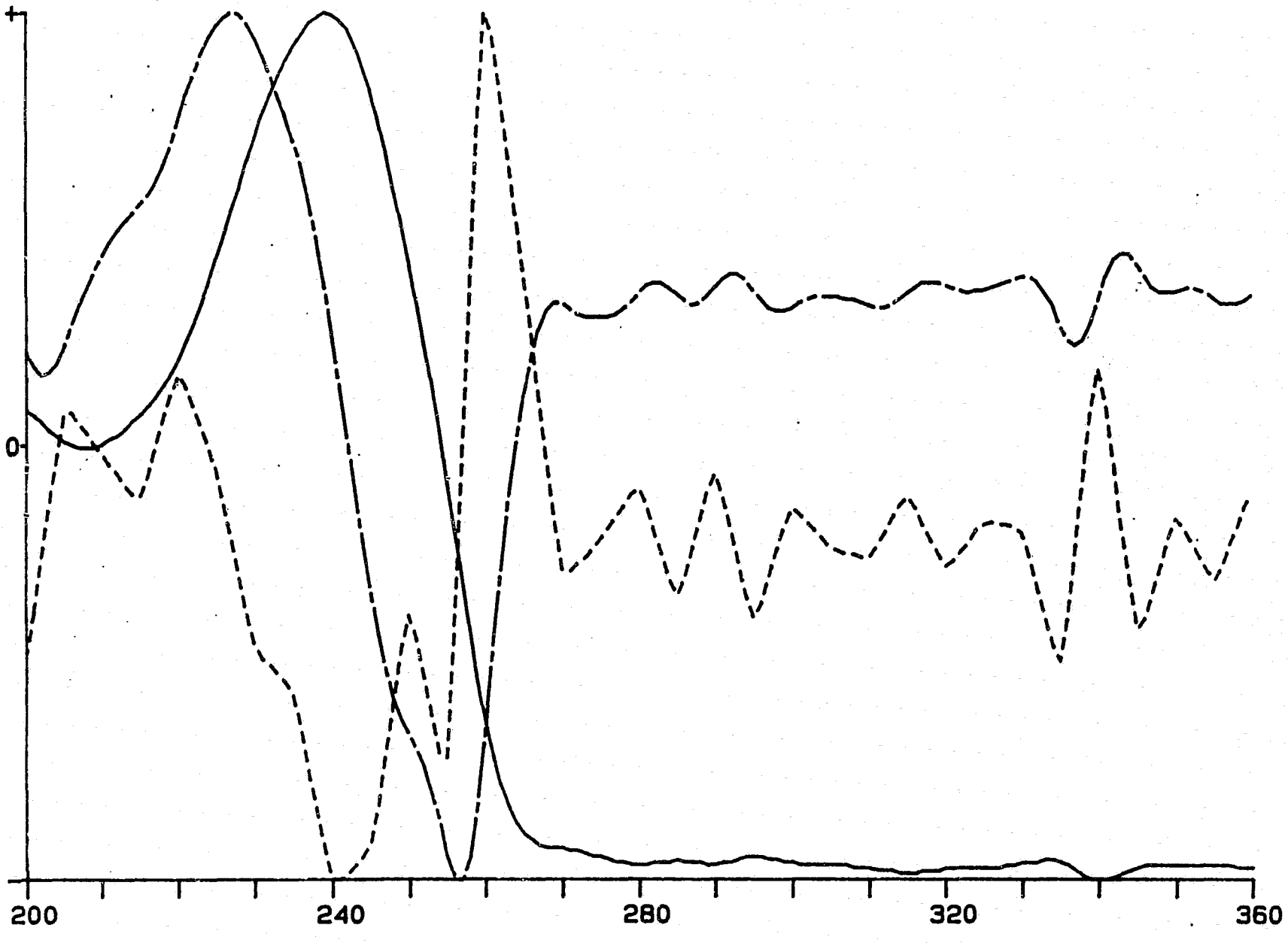

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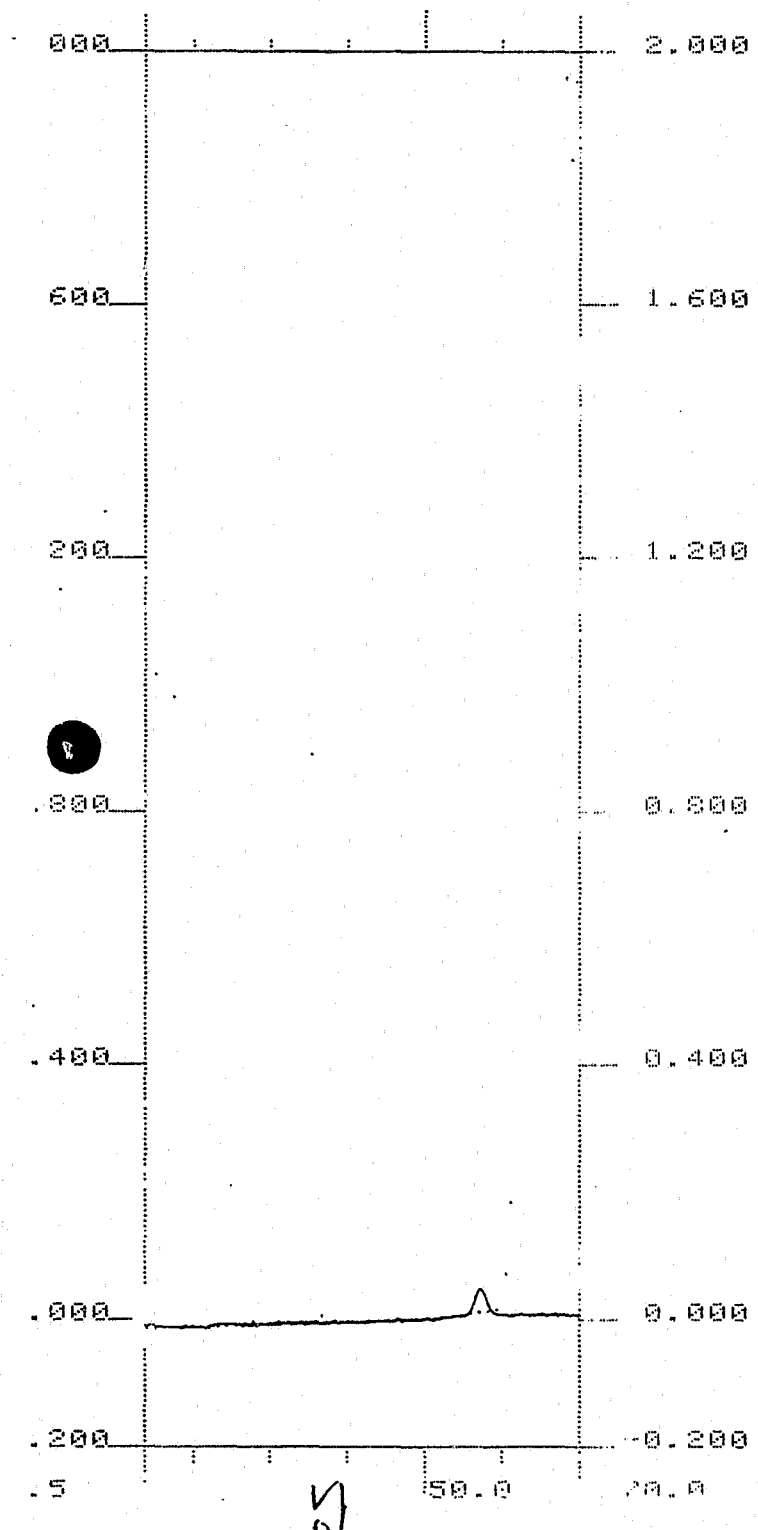

LANE NO. 2

NO.	Y POS.	AREA	MARK	%
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2	22.8	32.789	U	7.4
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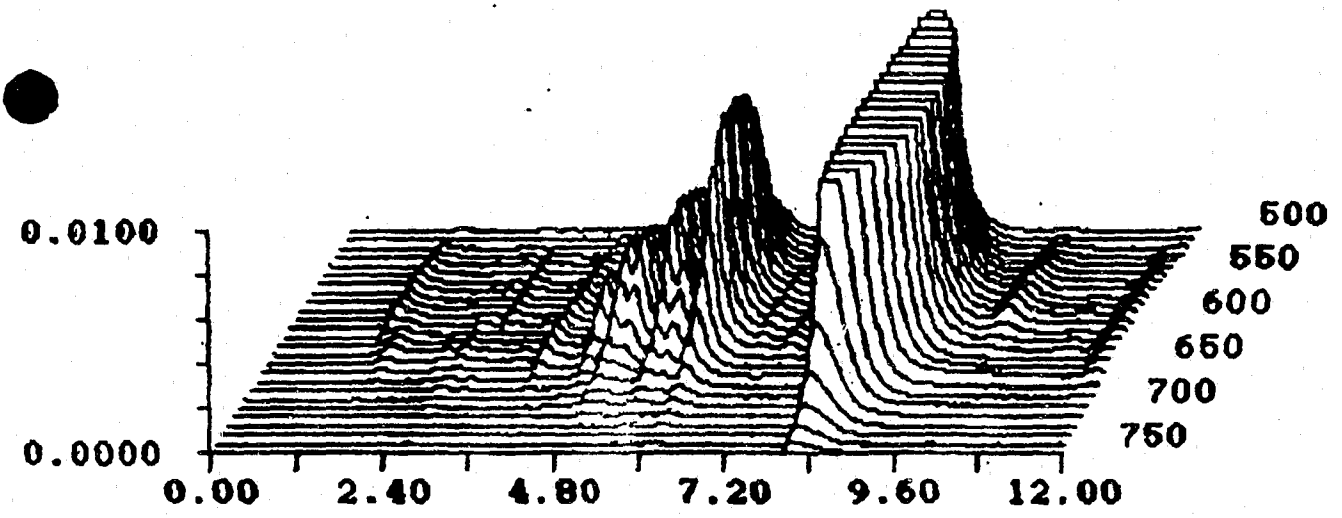

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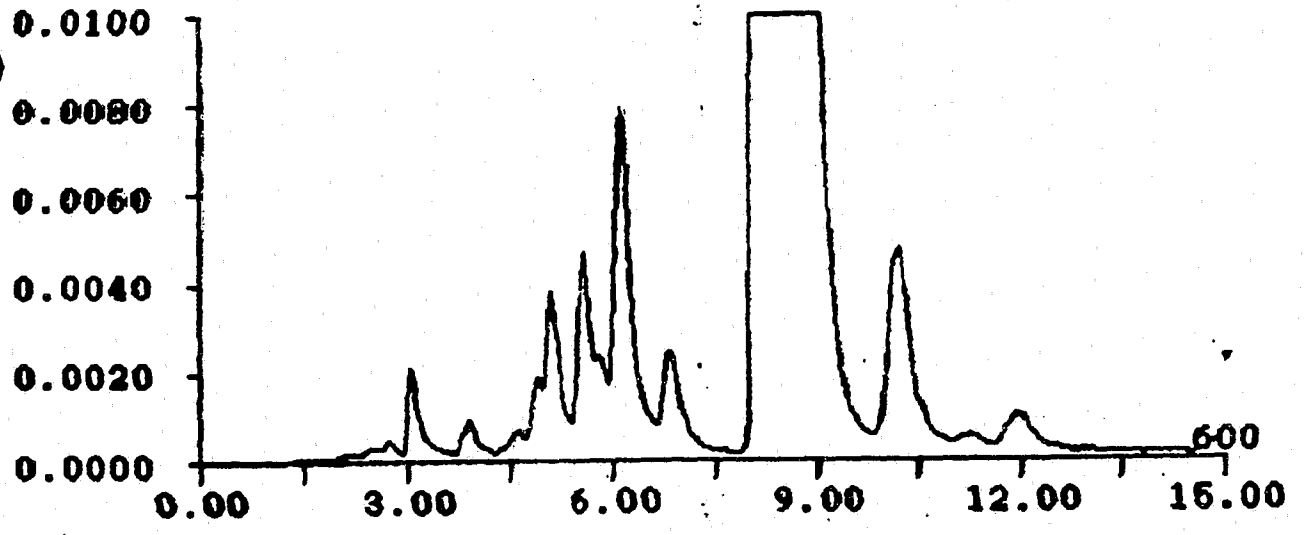
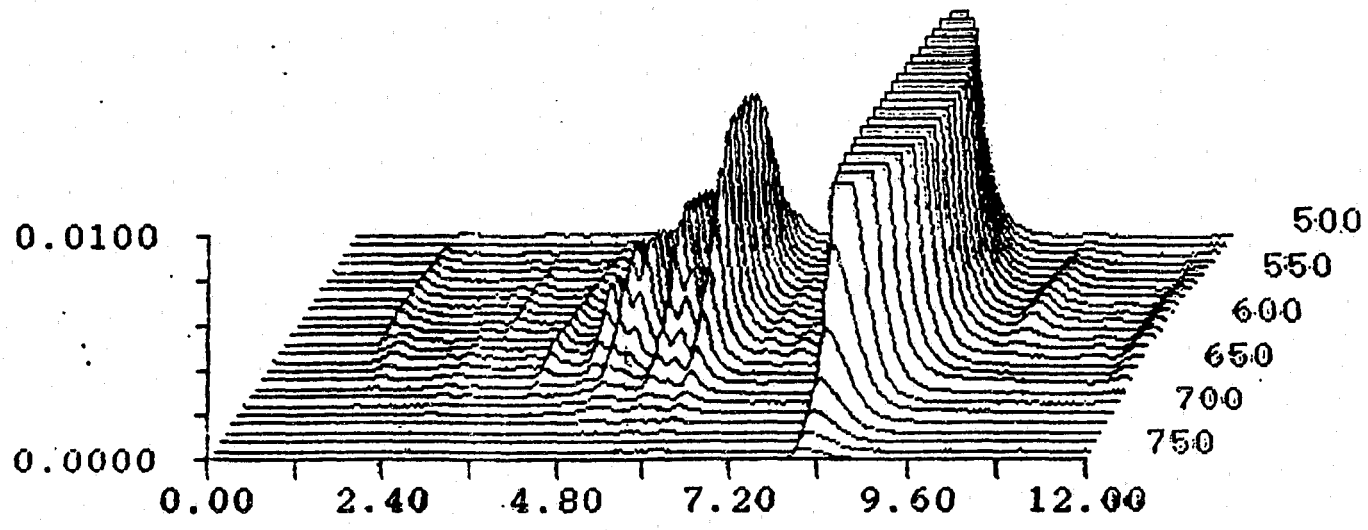


HPLC CHROMATOGRAM OF A YELLOW DISPERSE DYE EXTRACTED FROM A SINGLE POLYESTER FIBER.



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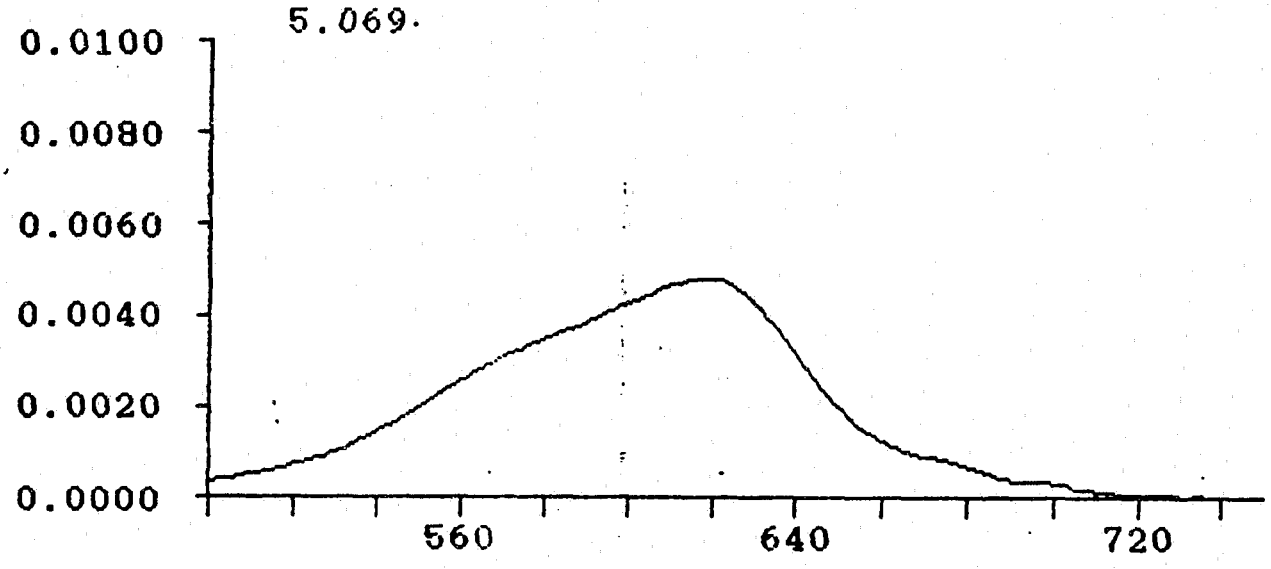

Spectra Display: \FOCUS\ A BFF

U.S. DEPARTMENT OF JUSTICE
OFFICE OF JUSTICE PROGRAMS
NATIONAL INSTITUTE OF JUSTICE

PROJECT REVIEW FORM

GRANT/CONTRACT #: 89-IJ-CX-0057

GRANT/CONTRACT TITLE:	GRANTEE/CONTRACTOR NAME AND ADDRESS:
Use of HPLC/Diode Array Detection for Analysis and Relative Dating of Inks and the Differentiation of Dyes	University of Illinois at Chicago Depart. of Grants and Contracts P.O. Box 6998 Chicago, Illinois 60680
PROJECT DIRECTOR AND ADDRESS:	**FUNDING LEVEL THIS PHASE:** / **TOTAL LEVEL:**
Ian Tebbett, Ph.D. Depart. of Pharmacodynamics (Same as Grantee)	$86,772 / $86,772
	PROJECT PERIOD THIS PHASE: / **TOTAL PERIOD:**
	9/1/89 - 8/31/90 / Same

PRODUCTS	TITLE AND AUTHOR	DATE SUBMITTED
1.	"The use of HPLC with Diode Array Detection for the Analysis and Relative Dating of Inks and The Differentation of Fiber Dyes", Final Report, by Ian Tebbett	8/24/90
2.		
3.		
4		

PROJECT MONITORS		FROM	TO
1.	Richard M. Rau	9/1/89	Present
2.		FROM	TO
3		FROM	TO

OUTSIDE REVIEWERS	NAME AND TITLE
1.	
2.	
3.	

STAFF REVIEWERS			
1.	Dr. Richard M. Rau	3.	
2.		4.	

PROJECT REVIEWER: R M Rau	DATE: 7/12/91
DIVISION DIRECTOR:	DATE:
OFFICE DIRECTOR: Richard S Laymon	DATE: 7/16/91

I. FINDINGS AND SUBSTANTIVE QUALITY

Grant Manager's Assessment Report

Provide a narrative assessment *not to exceed 200 words* describing the following: problem addressed and major objectives, accomplishments, activities undertaken, principal findings and documents produced. This report will be entered into the Grant Profile System (PROFILE). For further clarification of the requirements, see chapter 7 of the effective edition of OJP HB 4500.2.

This project developed innovative methods for the restoration and enhancement of blurred, grainy (noisy) and poor contrast images that were verified using photography from criminal cases. This project incorporated certain sensor nonlinearities into image restoration procedures, and developed methods for blur identification. Methods were also developed for simultaneous identification and restoration of spatially-variant blurs. Photographic images were scanned from film, slide, or print negatives and digitally converted to a computer screen for analysis. A user-friendly software package was developed implementing these novel and some other existing image processing algorithms/procedures -- gray scale modification by histogram specification, un-sharp masking, homomorphic filtering and adaptive versions. All these procedures were verified using both simulated and actual criminal photographic evidence. The Final Report is entitled: "The Use of HPLC with Diode Array Detection for the Analysis and Relative Dating of Inks and the Differentiation of Fiber Dyes."

89-IJ-CX-0057

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Dr Richard Rau,
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Criminal Justice Technology,
National Institute of Justice,
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Washington D.C. 20531.

June 3rd 1991

Dear Dick,

I apologize for the series of administrative errors which have resulted in you not getting copies of my reports. I am enclosing copies of the final report for the ink project together with a copy of an article which we have recently submitted to the Journal of Forensic Sciences, and the latest update on the drug project also with a paper recently submitted to the Journal of Forensic Sciences. As soon as these papers are accepted, I will forward the final copies to you.

In future I will make sure that I personally send you copies of everything.

As for the $15,000 returned as unobligated from the ink grant; it seems that money has been taken out of the wrong account. I will be contacting you when I have further information.

I look forward to seeing you in New Orleans, unless I can make it to Washington before that.

Yours sincerely,

Ian

Dr Ian R Tebbett
Director of Forensic Toxicology