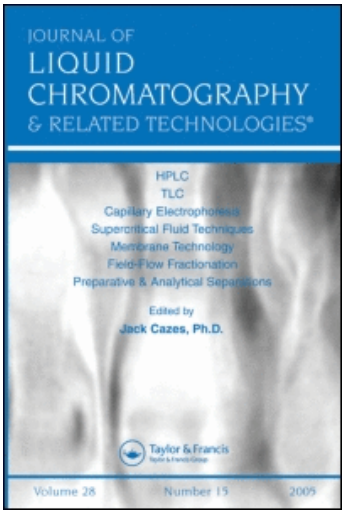

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Microcomputer-Assisted Acquisition and Reduction Of TLC Scanning Densitometric Data

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MICROCOMPUTER-ASSISTED ACQUISITION AND REDUCTION OF TLC SCANNING DENSITOMETRIC DATA

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ABSTRACT

The rapid microcomputer processing of quantitative TLC scanning data is described. The microcomputer, using BASIC language, is based on the Intel 8080A microprocessor IC and has 64K bytes of RAM. Data is acquired via a 16-bit integrating ADC. A TLC plate with up to 18 samples may be analyzed in as little as 5 minutes. Graphics capabilities include automatic analysis and depiction of peak heights and peak areas. Display of normal or log-normal plots of data for reference compounds and samples with least-squares linear regression is available. Graphics are directly accessible to permit independent plotting of data.

INTRODUCTION

Current systems for the acquisition of TLC scanning densitometric data can be interfaced with computing integrators; however, this practice is not entirely satisfactory since these integrators have restrictions as to rigidity of integration procedures and inability to manually overide integrations. Furthermore, proper laboratory practice and government regulations mandate the retention of large volumes of unprocessed data in support of analytical work. Such documentation is easier to use and more readily understood when available in a concise form. The automation of TLC data analysis addresses these problem areas. Microcomputers, user-programmable in a simple language such as BASIC, provide both analytical flexibility and condensed data storage.

EXPERIMENTAL

Microprocessor technology provides a means to automate TLC analyses, both conventional and high performance, in a package that is also fully compatible with GC and HPLC equipment. These demands for both data manipulation and instrumentation control have been satisfied by a microcomputer system developed by Woodward and Reilly[1] (Fig. 1). System components consist of an INTEL 8080A microprocessor IC, a 64-K byte random access memory, and a 16-bit integrating analog to digital converter for data acquisition. A television is used as a visual monitor while a flat-bed X-Y recorder serves as a printer-plotter. A

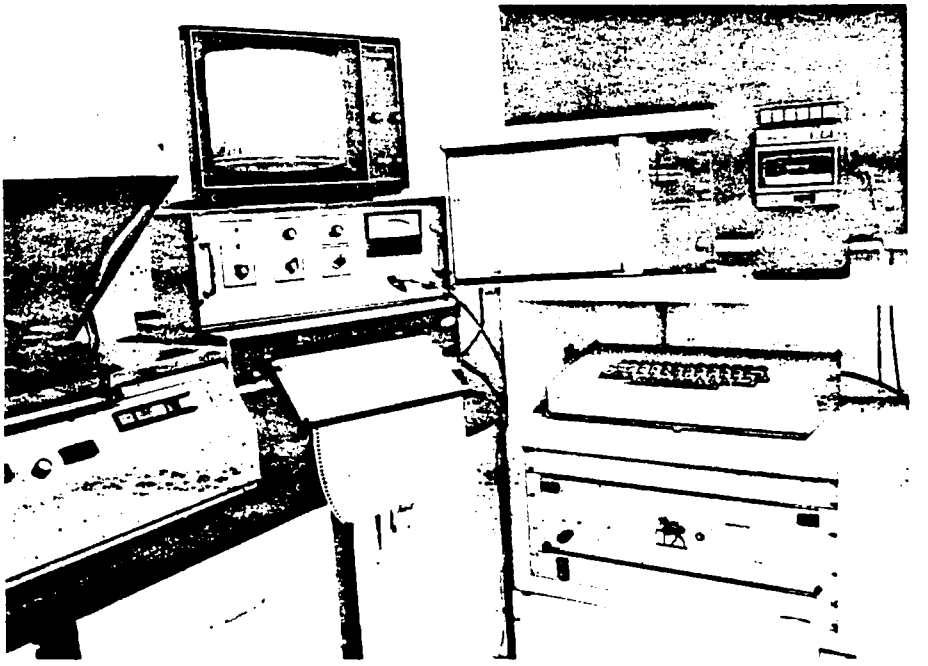


FIGURE 1

Microcomputer-based system described in text for data acquisition and reduction.

conventional cassette recorder is used for storage of data and programs on magnetic tape. The microcomputer is interfaced with a Schoeffel SD 3000 spectrodensitometer operating in the single-beam reflectance mode. Output to the microcomputer is obtained from the SD 300 density converter.

DISCUSSION

An essential portion of a successful instrument automation scheme is the development of a versatile computer program that is adaptable to the needs of the analyst. With this goal in mind a BASIC program has been developed in this laboratory to increase the data-handling efficiency of methods involving quantitative thin-layer chromatography.[2] The program will analyze and display data in any of several formats selected by the user. Data may be gathered in either absorbance or fluorescence modes. Plates may be scanned either parallel or perpendicular to the direction of development (termed normal-scan and cross-scan, respectively). Cross-scanning[3] permits the rapid analysis of TLC plates provided that the solute R_f's are consistent. Data obtained by cross-scanning is normally acquired continuously; however, the accumulation of data may be controlled manually to permit optimization of the detector. Optimization of the spectrodensitometer is achieved by manually aligning the scanning stage so that the light reflected from the analyte on the TLC plate elicits the maximum detector response. If both samples and standards are prepared in the same matrix, it is necessary to optimize the detector response of only the most intense spot in a series to be cross-scanned. If the standards are not in the same matrix, the response for both the most intense standard and sample should be optimized. Normal scanning requires optimization for each channel on the plate. During the scanning operation acquired data is displayed on the monitor. An operator-determined "scaling factor" normalizes this display to

utilize better the entire viewing area of the monitor. Automated analysis of unprocessed data provides number, retention time, area and height above baseline for each peak in tabular form on the monitor. Data can then be shown in the analog form in which it was acquired, with detected peaks numbered sequentially and marked to show areas of integration (Fig. 2). The program normalizes this display to the largest peak detected, thus, allowing accurate measurement of compounds in the low nanogram/spot range. Any peak displayed on the monitor may also be integrated manually. This data may be reproduced in tabular and analog forms using the X-Y recorder.

The calculated data may be further transformed to yield normal or log-normal plots of peak areas versus peak amounts (Fig. 3). The user determines which reference peaks and sample peaks are to be plotted. Appropriate dilution factors and reference peak amounts are next inputted, choices of normal or log-normal plot and visual display or copy are made, and the normalized reference data is plotted. An option is available to superimpose on the data the least-squares fit parameters and a line representing this regression. Additionally, sample data is fitted to the linear regression equation, and sample amounts and concentrations are depicted on the monitor.

The graphics portion of the program may be accessed to permit manual inputting of data, independently of the scanning data acquisition section. In this manner, plots may be obtained of any set of reference and sample data, such as drug plasma concentration-time curves (Fig. 4).

The automation system described herein accomplishes objectives frequently pursued by most modern analytical laboratories. Compliance with government regulations mandate the retention of large amounts of data to document analytical procedures. This task is simplified by storage of data on magnetic tape ensuring a means for rapid recall

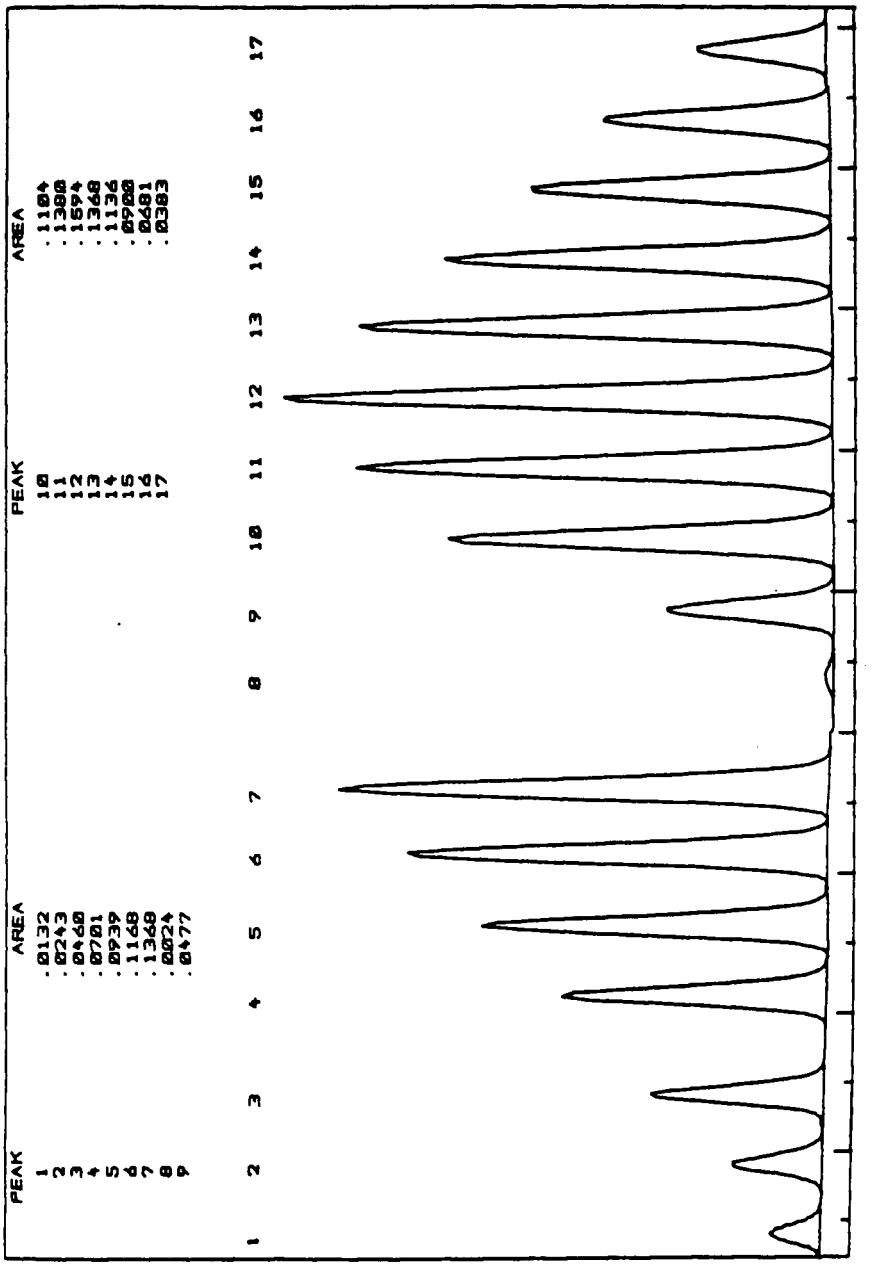


FIGURE 2. Representative plot of TLC data illustrating reference and sample peaks with corresponding peak areas obtained using system shown in FIGURE 1.

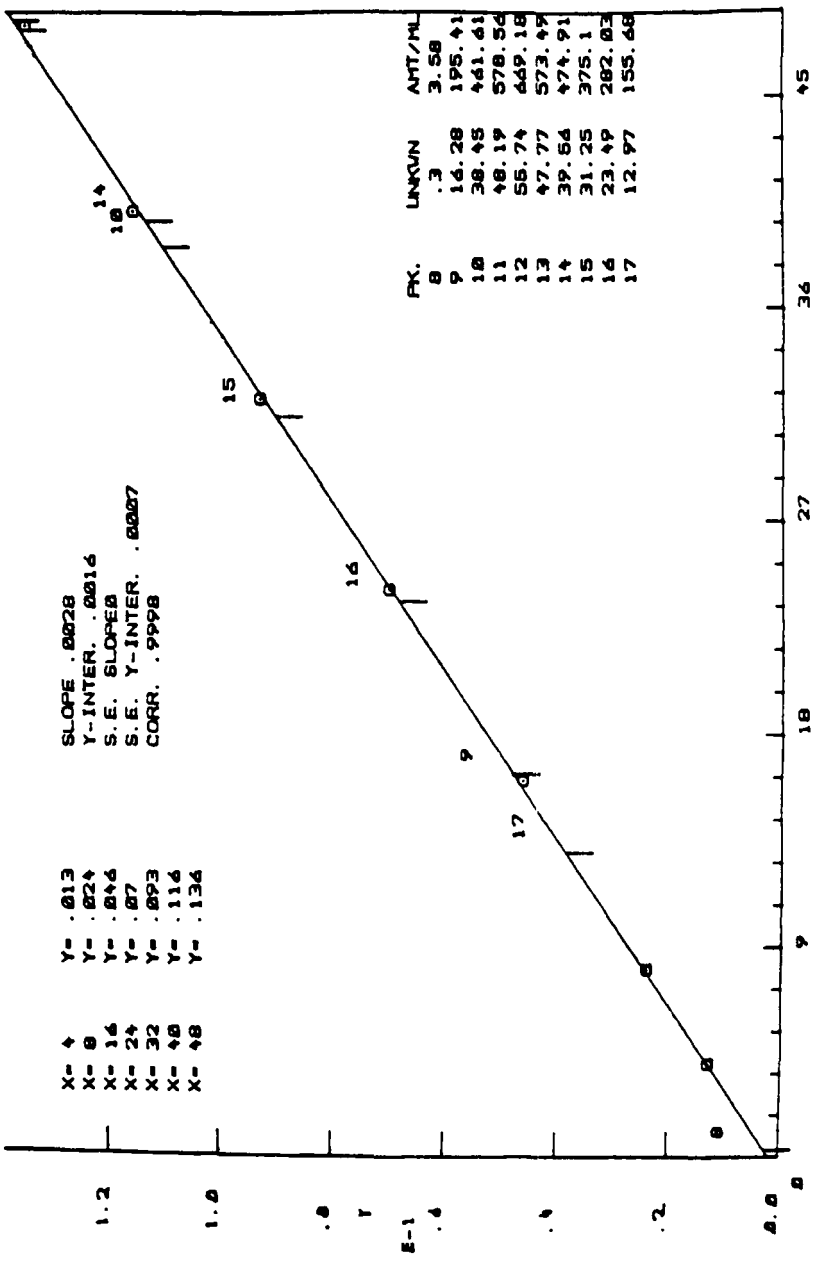


FIGURE 3. Plot of data from FIGURE 2 with "least-squares" linear regression fit and sample amount determinations.

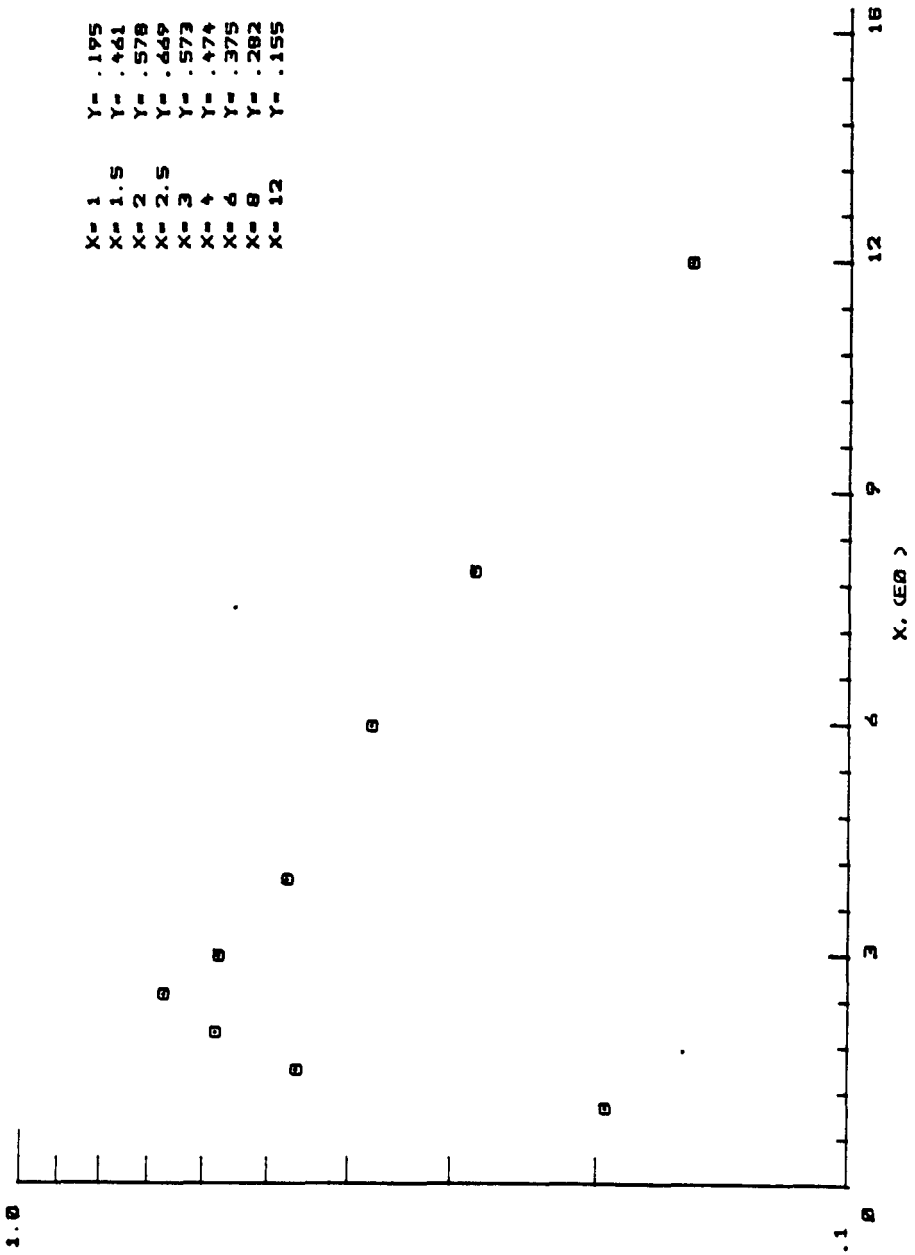
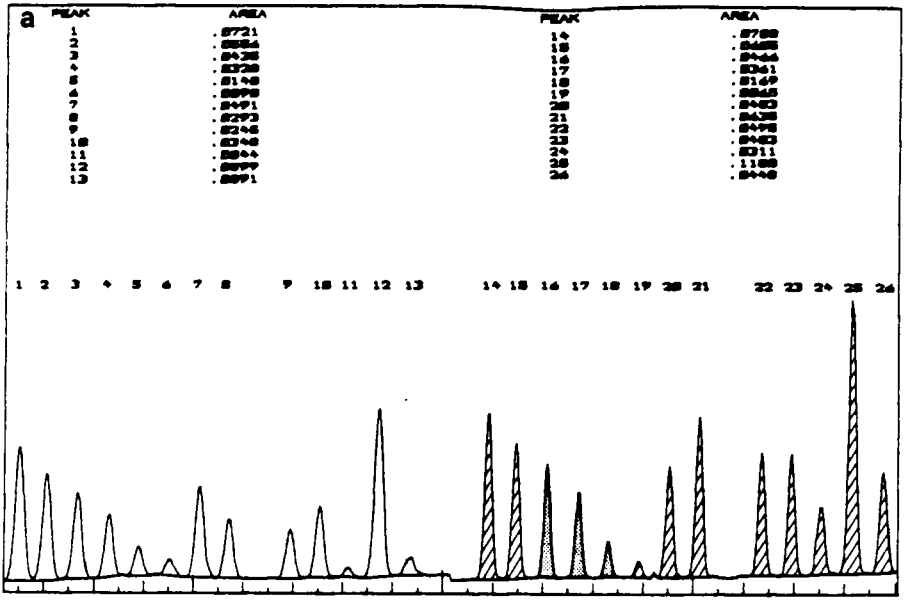
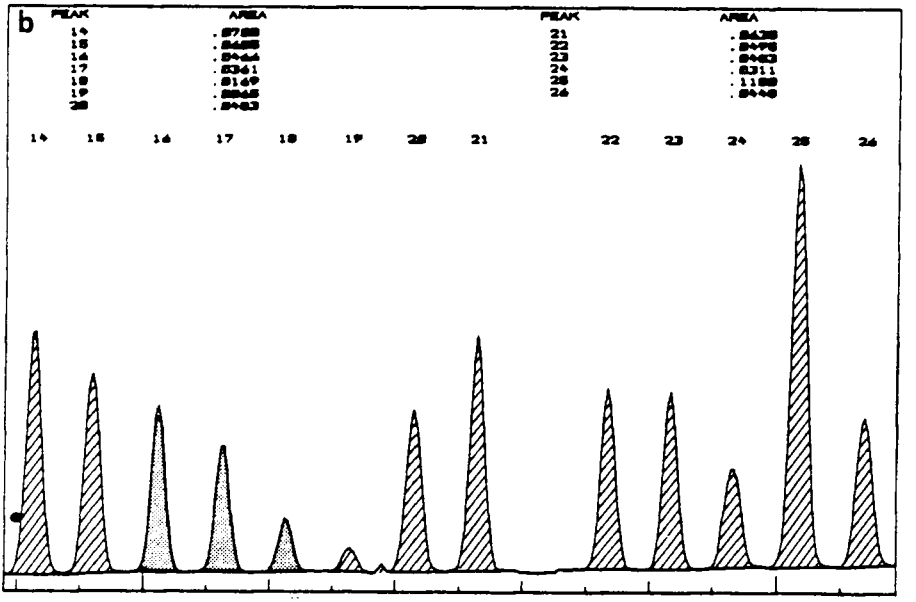


FIGURE 4. Variation of drug plasma concentration (μg/ml) with time over a period of 12 hours using data from FIGURE 3.

while providing a convenient method for permanent archival storage of data in a condensed form. The inclusion of processed data in the archival data set encourages good laboratory practice. Thus, depicted areas of integration and peak heights are available for future scrutiny. Furthermore, the unprocessed, or raw data, is not readily alterable, thereby precluding questions of data tampering. This system also provides reproductions of data via the printer-plotter that are suitable for direct inclusion in presentations, such as progress reports and publications. To suit individual requirements these formats may be altered through modification of the BASIC program.

The combination of this computer-assisted automation with the technique of cross-scanning conventional TLC plates has served to decrease analysis time. The observed increase in the instrumental sensitivity of this method over manual TLC scanning is derived from the ability to enlarge the portion of the chromatogram being analyzed (Fig. 5a-c). This capability has significantly increased the precision and accuracy of the instrumentation with which it has been used. Although the presence of reference and sample data simultaneously in the microcomputer memory makes TLC particularly adaptable to this data reduction scheme, other chromatographic equipment, such as HPLCs and GLCs, interfaces easily. Thus, the adaptation of automation techniques offers many advantages to the analytical laboratory. The extensive availability of microprocessor integrated circuitry makes feasible the development and widespread use of microcomputer-based automation systems.





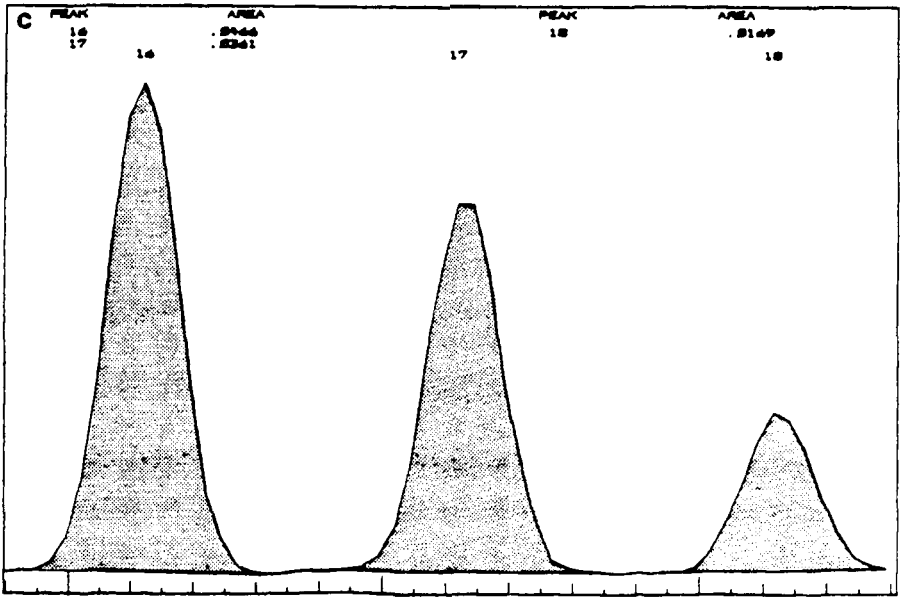


FIGURE 5

Successive enlargements of portions of a typical TLC chromatogram demonstrating potential for enhanced sensitivity of automated system described in text. (a) Display of peaks 1-26; (b) enlargement of section containing peaks 14-26; (c) further enlargement of section containing peaks 16-18.

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2. A facsimile of this program is available from our laboratory upon request.

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