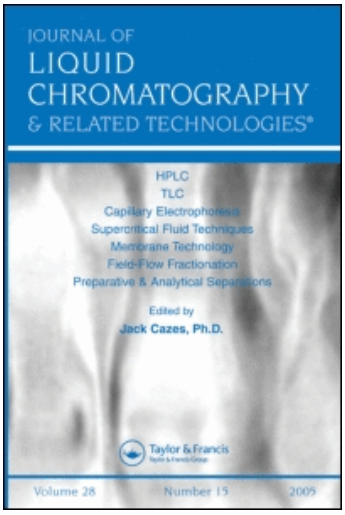

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LAPTOP CHEMISTRY: A COMPACT, PORTABLE THIN LAYER SCANNER

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ABSTRACT

We introduce a field portable Thin Layer Chromatography (TLC) plate scanner based on digital image processing hardware. The TLC scanner module consists of a Charge Coupled Device (CCD) imaging detector, a Xenon strobe light source, and the necessary hardware to capture and process video images. The instrument is bundled with a laptop computer to form a compact instrument the size of a 6 inch thick briefcase.

To automate analysis, software has been developed for (a) subtraction of dark noise, correction of variations due to pixel sensitivity and spatial nonuniformity of source intensity, (b) automated lane finding, (c) comparison to standards for on-plate calibration, and (d) peak finding and integration. Response to analyte concentration is linear with a detection limit of 40 ppb for sulfamethazine spotted on a plate, with correlation coefficient of 0.994.

INTRODUCTION

Adulteration of meat and foodstuffs by chemical residues represents a source of increasing concern. Considerable research has been focused on development of methods for residue analysis. However, most of these methods require complex instrumentation as well as skilled personnel. In contrast, Thin Layer Chromatography (TLC) is a technically simple method that has proven utility for residue analysis (1,2,3). TLC has many

advantages: low cost, good separation efficiency, high throughput (many samples can be analyzed on the same plate), and suitability for field screening.

The United States, Canadian, and Ireland Departments of Agriculture have implemented the Sulfa-On-Site or SOS test for the routine monitoring of sulfonamide residues in pork products. The SOS test is based upon a channeled, silica TLC plate with a preadsorbant zone (4,5,6). The determination is made semi-quantitatively by eye in the field by comparison to on-plate standards, or quantitatively by mechanical scanners in the regional labs. These scanners tend to be slow, expensive, and the results can be user dependent. In contrast, we have previously shown that TLC plates could be reproducibly quantitated using video based scanners (7,8). The advantages of this approach include elimination of mechanical components, rapid data acquisition, and ability to use sophisticated image processing algorithms for quantitation. In addition, video densitometry is readily adaptable to portable instrumentation, making it possible to do complex analyses in remote locations, i.e. a grain silo, or a slaughterhouse.

EXPERIMENTAL

In the SOS test, 20μL of urine taken from a swine carcass is spotted directly onto the preadsorbant zone of a channeled, silica, TLC plate (Whatman LK6D) with a calibrated micro-capillary. Six samples and 2 standards, one at 1.3 ppm sulfamethazine and another at 0.4 ppm, are spotted onto the 10 lane plates, with the two outside lanes left empty. The samples are spotted on the plate, dried, and then concentrated by elution to the top of the preadsorbant zone in methanol. The solvent is evaporated and the separation is carried out by eluting to 3 cm past the preadsorbant zone in ethyl acetate. Once again the solvent is evaporated and the plates are then sprayed with a solution of 30 mg fluorescamine in 250 ml acetone. Fluorescamine reacts specifically with the primary amine group common to sulfonamides and yields a fluorescent moiety (9). The fluorescence

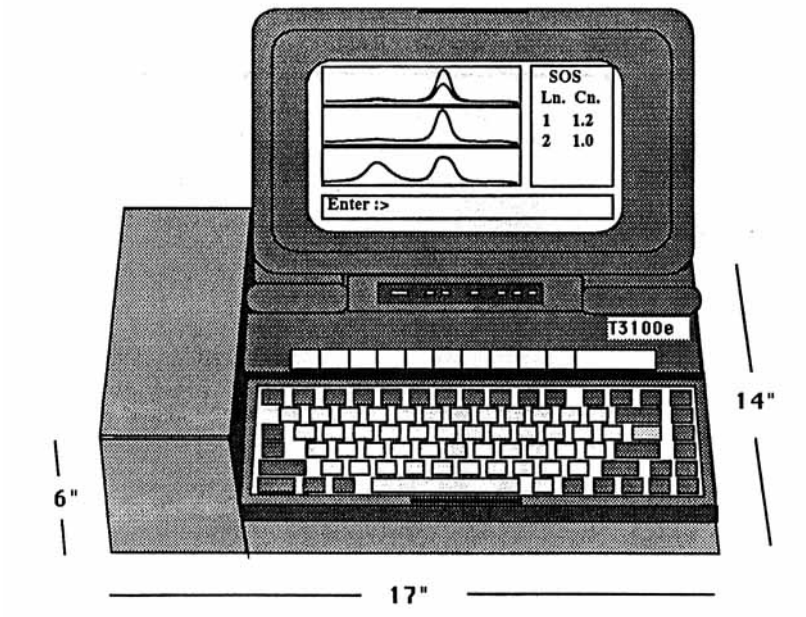

Figure 1. Laptop Computer and Hardware module

characteristics of the sulfonamide/fluorescamine moiety show maxima for λ_{ex} at 410 nm and λ_{em} at 510 nm. Thus, in order to select the proper wavelengths for analysis, an 80 nm (FWHM) band pass filter centered at 510 nm (Corion Corp.) was placed in front of the detector and excitation wavelengths were selected by placing a UG11 Schott Glass filter (Rolyn Optics) in front of the light source.

The scanner system configuration is that of a laptop computer coupled to a hardware module as shown in Figure 1. The laptop computer used was a Toshiba T3100E(12Mhz clock rate, 80286 processor, 20 Mb hard disk). The hardware module houses the detector system, light source, and sample holder, coupled to a platform that contains the support electronics necessary for operation. A layout of the sample compartment is shown in Figure 2. The detector system consists of a CCD camera and an 8-bit A/D converter

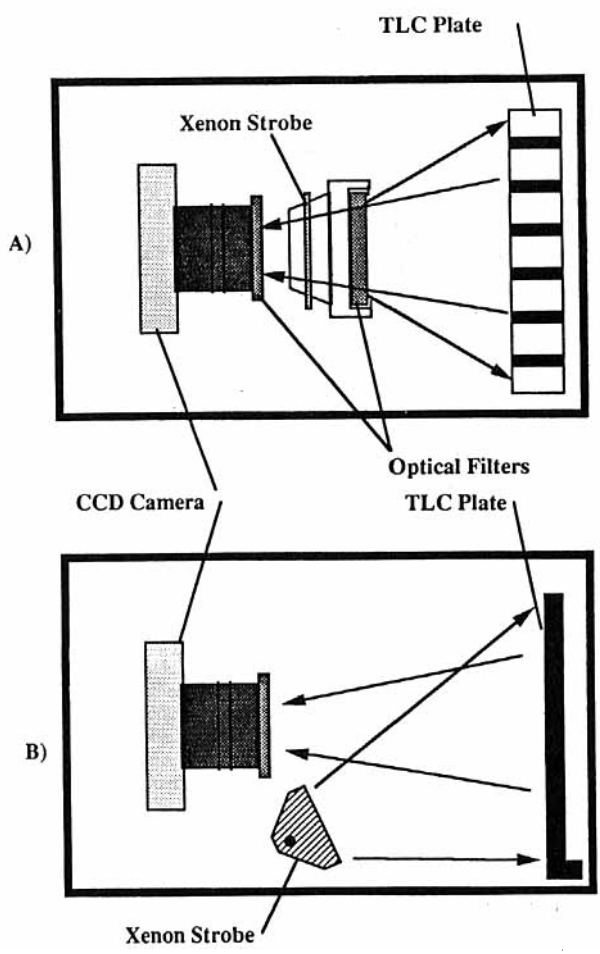

Figure 2. A) Top View of Sample Compartment . B) Bottom view.

(Electrim Corp. Model EDC-1000). We chose this detector system because of its sensitivity and low cost. The EDC-1000 utilizes the Texas Instruments CCD imaging chip (TC-211), which provides an array of 31,680 pixels arranged as 192 columns x 165 rows. The camera is fitted with a 4.8 mm f/1.8 lens (D.O. Industries) and placed such that an image of the entire TLC plate fills the detector array.

The light source is a Xenon flash lamp from a commercial photo-strobe (Achiever Mdl. 113A) equipped with a built-in reflector. This source is inexpensive, and capable of relatively high power output. The home constructed power supply for the strobe is a circuit capable of supplying 20 joules/flash at repetition rates from 0-10 Hz. Wavelength selection is accomplished by means of a filter positioned immediately in front of the strobe unit. The strobe trigger and camera are controlled by the computer via the serial port. For fluorescence measurements, the emission wavelengths are selected by placing the appropriate band pass filter in front of the camera lens. The pattern of the flash illumination on a plate is stable to within 3%. However, the illumination pattern is non-uniform across the surface of the plate, due to the placement of the strobe. In order to correct pixel by pixel for this non-uniformity, a ratio is calculated of the fluorescence image taken at the excitation wavelength, $\lambda_{ex.}$ against a reflectance image taken with the excitation filter removed. This is done without moving the TLC plate, thus creating a flat illumination field. In order to obtain a reference image of comparable intensity, an O.D. 4.0 neutral density filter was placed in front of the strobe.

In order to automatically locate the lanes, a "lane finding" algorithm was developed using the high contrast reference image. Several rows of this image are summed into a single vector to increase the signal-to-noise ratio over that of a single row. The derivative of this vector was taken. The zero crossings of the derivative clearly mark the transitions between lanes on a channeled TLC plate. Figure 3 shows a schematic diagram of the lane-finding algorithm.

RESULTS

Camera linearity was evaluated by exposing the camera to a uniform surface subject to continuous illumination and interposing neutral density filters between the lens

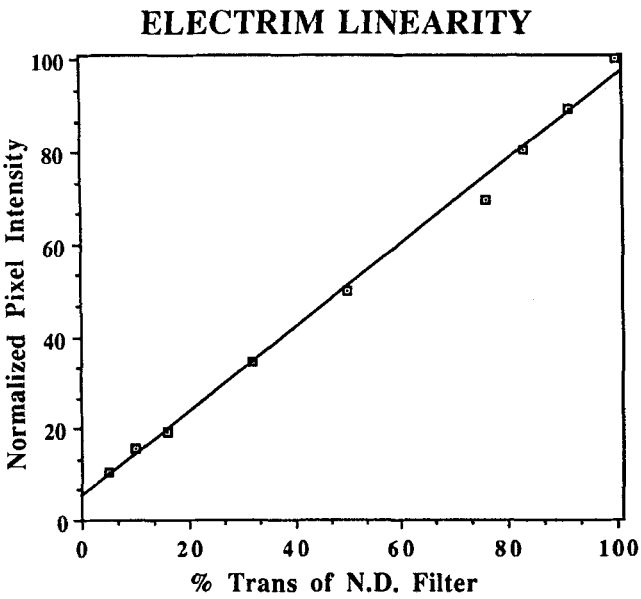

Figure 3. Each datum is the mean value of 5 image measurements. Each image value is a 16 frame ave. with 100 pixels selected. 90 msec integration, f/1.3.

and the surface. The response shown in Figure 4 is quite linear with a correlation coefficient of 0.996. Error bars are not visible on the scale of the graph.

To evaluate the scanner's linearity of response to analytes, and the variability between plates, 5 plates were spotted with a series of sulfamethazine (SMZ) standards (Aldrich Chemical) in methanol, whose concentration ranged from 0.04 to 2.0 ppm. The area under each of the standard peaks was determined by subtracting a baseline drawn from the beginning of a peak to the end of the peak, and summing the intermediate values. Peak endpoints were determined using a derivative slope algorithm. A regression of these areas against the standard concentrations results in R^2 values ranging from 0.999 to 0.971. Standard errors of estimate ranged from 0.036 to 0.14 ppm respectively. The

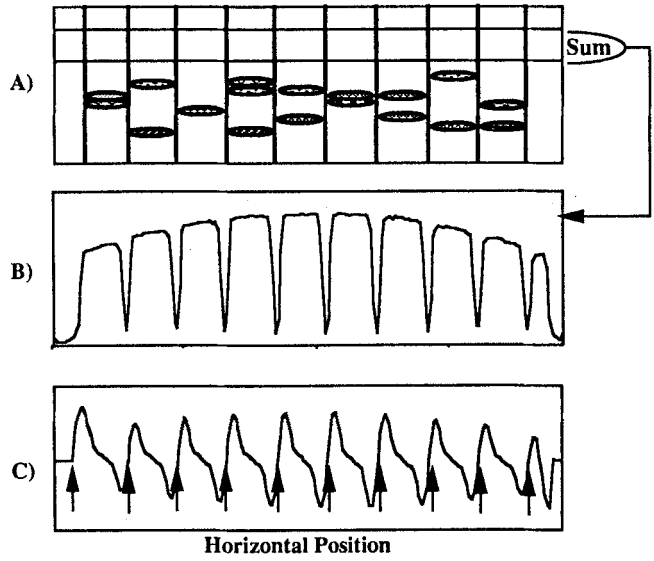

Figure 4. Schematic Representation of Lane Finding Algorithm. Arrows Indicate Detected Edges. A) Image of TLC Plate, B)Vertical Sum, C) Derivitive of Vertical Sum.

uncertainties in this assay are believed to arise largely from the manual manipulations of the protocol, i.e. sample spotting, solvent evaporation, reagent application, and not to instrumental signal-to-noise limitations. A calibration curve for the combined data sets is shown in Figure 5. This calibration spans the normal regulatory range for SMZ and is not limited by the dynamic range of the instrument, as evidenced by comparison to Figure 4.

In a separate experiment, we evaluated the intra-plate reproducibility of the assay. Three plates were spotted with 8-20 μL aliquots of 1.5 ppm sulfamethazine, and 3 other plates were spotted with 0.5 ppm sulfamethazine. The plates were eluted and developed using the protocol described previously. The Coefficients of Variation (CV) of the

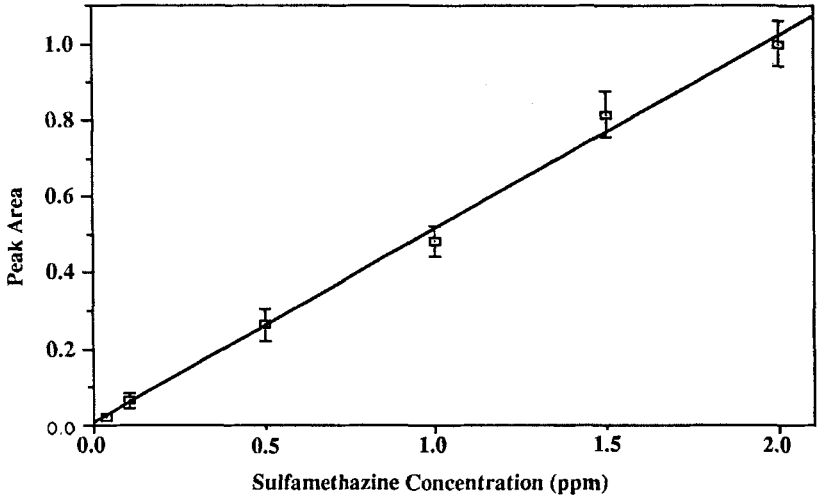

Figure 5. Calibration Curve. Error Bars = 1 Sigma.

measured peak areas for the 1.5 ppm and 0.5 ppm solutions are %10.2 and %19.0 respectively. The finding that uncertainties within and between plates is equal confirms the earlier assertion that the errors arise largely from the sample application, development and staining rather than instrument noise.

One potential problem with the TLC based assay is inadequate resolution. Frequently there is more than one drug present. For example, a related drug, sulfadimethoxine (SDM) nearly co-elutes with the primary analyte, sulfamethazine (SMZ). Thus, its presence is a potential interferent to accurate quantitation. In order to determine the magnitude of the error associated with quantitating sulfamethazine in the presence of sulfadimethoxine, several drug mixtures were spotted and chromatographed, with the peak deconvolution accomplished using the vertical drop method. Figure 6 shows the smoothed chromatographic traces of three different SMZ-SDM mixtures. The SMZ/SDM

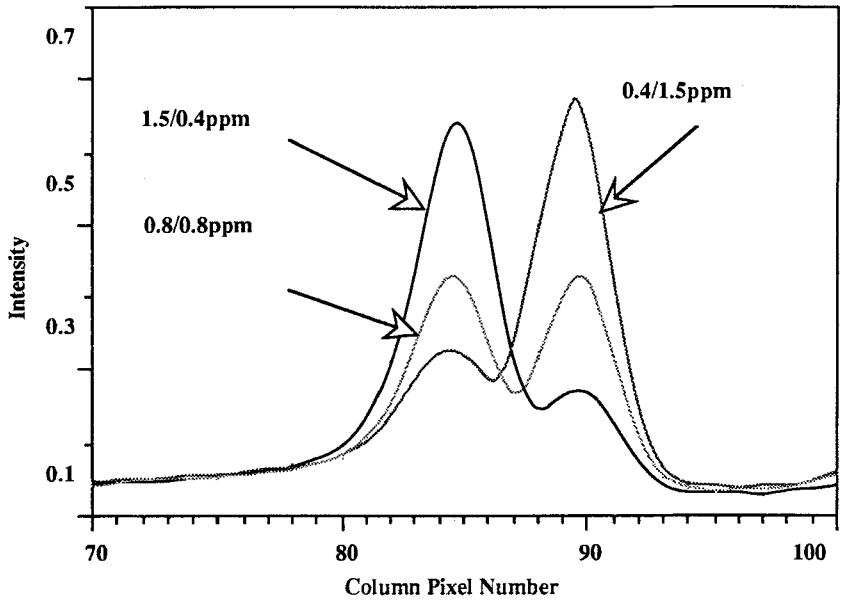

Figure 6. Chromatographic Traces of Sulfonamide Mixtures. Ratios Indicate SMZ/SDM Concentrations.

Table I
Sulfonamide Mixtures

Mix #	Conc. Ratios SMZ/SDM ppm	Peak Areas SMZ	SDM	Predicted Conc. SMZ ppm	SDM ppm
1	1.5/0.4	2.11	0.43	1.42	0.29
2	0.4/1.5	0.67	1.95	0.45	1.31
3	0.8/0.8	1.18	1.03	0.79	0.85

concentration ratios are 1.5/0.4, 0.4/1.5, and 0.8/0.8, where SDM elutes first. The quantitation results, based on-plate SMZ standards, are shown in Table I. Note that the SDM peaks were quantitated using the SMZ calibration. The results show that the reproducibility for overlapping peaks is nearly within the %10 CV for the single peak measurement. These results are also consistent with the use of the vertical drop method of peak area determination. We feel that results for overlapping peak quantitation could be improved by a reduction of the spotted sample volumes. The 20 μL aliquots overload the channeled column, resulting in spots that tend to concentrate at the edges of the channel. This can lead to peak broadening which may cause large errors, especially in the case of overlapping peaks.

CONCLUSIONS

We have shown that quantitation of TLC plates using field portable digital imaging technology is feasible. Moreover, sensitivity and accuracy are more than adequate for the SOS test. Other possible applications include a host of environmental/residue methods. Moreover, hardware enhancements such as a cooled CCD camera and a higher resolution imaging chip, should further the capabilities of the system by increasing the sensitivity and spatial resolution. With these improvements, the performance of the system should rival that of the state of the art in scanning densitometers.

The image processing approach is applicable to many other types of analyses such as 96 well ELISA plates, gel electrophoresis, zones of inhibition, etc., thus, the laptop densitometer and its imaging technology seem assured of an expanding role in the analytical community.

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