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QUANTITATIVE THIN-LAYER CHROMATOGRAPHY BY LASER PYROLYSIS AND FLAME IONIZATION OR ELECTRON-CAPTURE DETECTION

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SUMMARY

A new quantitation method, laser pyrolysis, is demonstrated for thin-layer chromatography (TLC). No spray reagent or "color" developing process is necessary for detecting any organic compound. A complete analysis including sample introduction, separation and detection takes less than 20 min. Two amino acids, serine and phenylalanine, and two pesticides, *p,p*-DDT and methoxychlor were used as the test samples. The sensitivity and linearity compare favorably to conventional densitometry. The detection limit for phenylalanine with flame ionization detection was 100 ng and for methoxychlor with electron-capture detection was 20 ng. This technique combines the advantages of the separation power of TLC and the broad spectrum of detection methods of gas chromatography.

INTRODUCTION

Thin-layer chromatography (TLC) is a simple, rapid and versatile separation technique. Features of two-dimensional separation and multiple sample handling have also contributed to its widespread application. TLC readily provides qualitative results. For quantitative determinations, densitometry, fluorimetry, fluorescence quenching, visual comparison, spot area measurement, and radioactive methods are representative of the methods currently available[1].

Laser-based fluorimetry is the most sensitive technique. Detection limits are often in the 1–10 pg range[2]. Fluorescence detection is however limited to those compounds which fluoresce or can be conveniently derivatized to become fluorescent. Densitometry is the most common quantitation method in TLC. Detection limits with commercial scanners and high-performance plates are typically at the nanogram levels for compounds that absorb visible or UV light strongly. With the recent development of a laser photo-acoustic densitometer[3,4], detection limits of 7.5 pg for α-ionone and 170 pg for orange G have been reported. All the quantitative techniques except radioactive methods are highly dependent on the optical properties (absorbance or fluorescence) of the analytes. Sensitivity varying over several orders of magnitude is observed.

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Many interesting applications of TLC are for "colorless" or very weakly absorbing compounds, *e.g.*, hydrocarbons, lipids, pesticides, carbohydrates, amino acids, proteins and glycols. Normally a "color" developing or spraying process is needed. "Color" developing processes are often undesirable. Heating and even UV radiation are usually involved to promote the reaction. For example, one determination of amino acids with densitometry was described as follows: spray with ninhydrin reagent at a distance of 30 cm from the plate; heat it in an oven for 15 min at 60°C; and place it in a dark cupboard for 4 h before scanning[5]. Two spray reagents and two heating procedures were proposed recently for amino acids analysis with a limit of detection of 0.5–1.0 μg[6]. Quantitative results rely on the choice of spray reagent, spraying skill, heating temperature and heating time. Reproducibility is therefore poor. Even after spraying, optical detection on an opaque and intensely light scattering TLC plate is a difficult task. The Kubelka-Munk correction is often needed for nonlinear effects, although implementation is not difficult with the help of personal computers.

In this paper we describe a new quantitative TLC method: laser pyrolysis scanning (LPS). This technique is simple, rapid, highly instrumental, and has little dependence on the optical properties of the analytes. There is no need for spray reagents and "color" developing, and is applicable to all organic compounds. Briefly, a TLC plate after separation of the analytes is irradiated with an infrared laser to produce a high-temperature spot. The analyte is thus pyrolyzed and swept into a flame ionization detector or an electron-capture detector by a carrier gas.

EXPERIMENTAL

Apparatus

The schematic arrangement of laser pyrolysis scanning with a flame ionization detection (LPS–FID) system is shown in Fig. 1a. A CO_2 cw laser (Molectron Model C250, Sunnyvale, CA, U.S.A.) was used and the laser beam was focused to 2.5 mm on the TLC plate with a 1.0-m concave mirror. Either a flame ionization detector or an electron-capture detector [both were dismounted from a Model 550 gas chromatograph (Tracor, Austin, TX, U.S.A.)] was directly connected to one end of the cell. The carrier gas used was hydrogen–helium (1:2) for FID and argon–methane (9:1) for electron-capture detection (ECD), and the flow-rate was 80–100 ml/min. The signal was collected by an integrator (CI 3000).

Fig. 1b shows the details of the pyrolysis cell. The cell was made of copper and has a 70-mm long, 12-mm wide, and 7-mm high chamber to accommodate a 50 mm × 12 mm TLC plate. The open end of the cell can be closed with an O-ring seal. A potassium chloride window (50 mm in diameter) was attached to the cell with epoxy (Eccobond, Waltham, MA, U.S.A.).

Chemicals

The test compounds were phenylalanine, serine, *p,p*-DDT, and methoxychlor. These were obtained from Aldrich and used as received. The amino acids were dissolved in water (pH = 4 with nitric acid) and the pesticides were dissolved in methanol–hexane (95:5). All solvents used were high-performance liquid chromatographic (HPLC) grade. Samples at various concentrations were prepared by dilution with the solvent used.

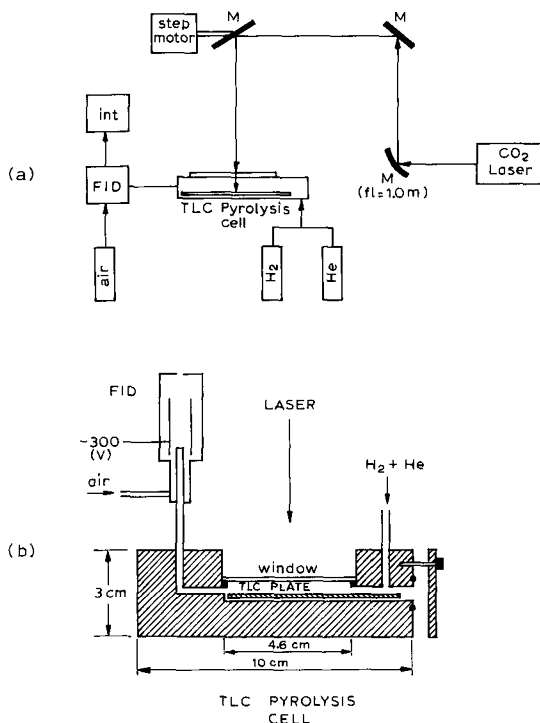


Fig. 1. (a) Schematic diagram of quantitative TLC with laser pyrolysis scanning and flame ionization detection. (b) Details of the laser pyrolysis cell. M = mirror, int = integrator.

TLC separation

Two kinds of silica gel glass-backed plates were used, one with calcium sulphate binder (Alltech, Deerfield, IL, U.S.A., Adsorbsil HPTLC) and the other without binder (Alltech, Adsorbsil-plus, soft layer). Plates were cut to the desired sizes with a glass cutter. The soft layer plate was cleaned with methanol and the plate with binder was treated with concentrated nitric acid overnight, washed with pH 10 buffer and then with deionized water, dried under a 200-W infrared lamp and passed quickly over a flame. Plates were stored in a beaker filled with nitrogen.

Mixtures of the test compounds were applied as 0.2-μl spots with a 10-μl Hamilton microsyringe. Plates were developed in a 30-ml beaker covered with aluminium foil, and dried under an infrared lamp in a nitrogen-filled beaker for about 3 min to evaporate the solvents. The plate was scanned as soon as possible. Both flame ionization and electron-capture detectors were stable within 2 min after the TLC plate was introduced.

Scanning

The laser beam was controlled by a rotating mirror driven by an EPC-012 stepping motor (Hurst, Princeton, IN, U.S.A.). A 46-mm length of the TLC plate was scanned with a speed of 20 mm/min in a direction opposite to the carrier gas-flow. The laser power varied from 1.0 to 5.6 W.

RESULTS

Flame ionization detection

Fig. 2 shows the thin-layer chromatogram of LPS–FID for two amino acids, serine and phenylalanine, both *ca.* 1.0 μg. They were separated on the TLC plate with binder, with water as developing solvent. R_F values were 0.85 for serine and 0.7 for phenylalanine. The response factors were in close agreement with the carbon content of the amino acids, which was expected for FID. The laser power used was 3.5 W.

The dependence of the FID signal on laser power has been examined and is shown in Fig. 3. Higher laser power gives more efficient pyrolysis and shows large FID signals. However, the background is also increased. For the best signal-to-noise ratio, 3.5 W was used. The limit of detection (LOD) is 100 ng for phenylalanine and 500 ng for serine. Linear calibration curves were obtained from the detection limits to 16 μg.

Electron-capture detection

The mixture of the organochlorine pesticides, *p,p*-DDT and methoxychlor, was separated on the TLC plate without binder. The developing solvent was hexane–methanol (99:1). R_F values were 0.6 for *p,p*-DDT and 0.1 for methoxychlor. Fig. 4 shows the thin-layer chromatogram of the pesticides with LPS–ECD. The laser power used was 2.5 W. The dependence on laser power is shown in Fig. 5. For laser powers above 2 W, the samples were almost completely pyrolyzed due to their low thermal stability. The LODs are 20 ng for methoxychlor and 50 ng for *p,p*-DDT. The response for methoxychlor was linear from 50 ng to 2.5 μg and that for *p,p*-DDT was linear from 200 ng to 2.5 μg. As one approaches the LOD, the signal was found to become non-linear and decrease rapidly.

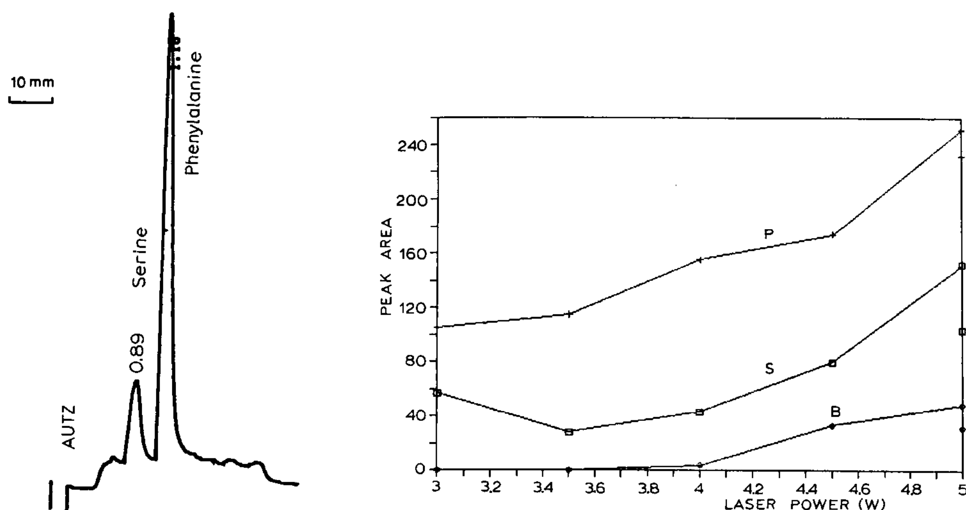
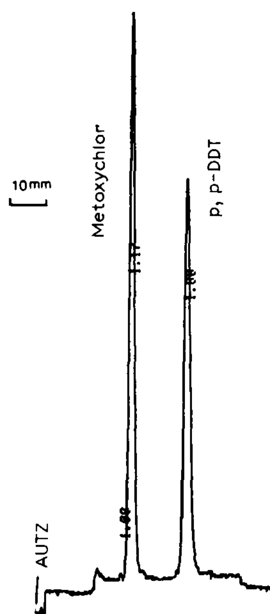


Fig. 2. Thin-layer chromatogram of two amino acids with LPS–FID. The sample contains 0.96 μg serine and 1.02 μg phenylalanine. Laser power, 3.5 W; AUTZ = auto-zero to denote start of scan.

Fig. 3. Laser power dependence of LPS–FID. S = serine, 0.96 μg; P = phenylalanine, 1.02 μg; B = background taken from the largest noise peak.



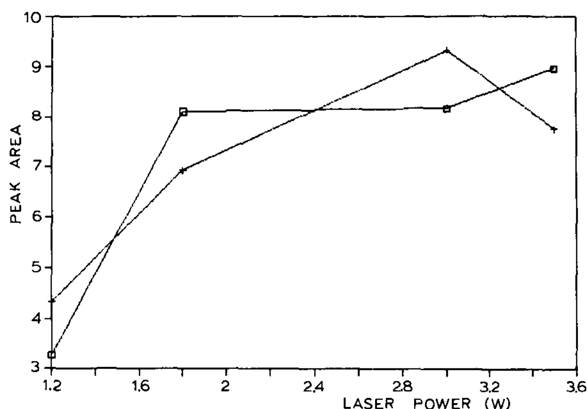


Fig. 4. Thin-layer chromatogram of two pesticides with LPS–ECD. The sample contains 220 ng *p,p*-DDT and 270 ng methoxychlor. Laser power: 2.5 W.

Fig. 5. Laser power dependence of LPS–ECD. (□) *p,p*-DDT, 440 ng; (+) methoxychlor, 530 ng.

DISCUSSION

The sensitivity and the linearity obtained are not very impressive compared to fluorescence[2] or photothermal methods[4]. However, they are comparable to, or even better than those obtained with conventional densitometers. In densitometry, the detection limit of amino acids was 0.1–0.5 μg with ninhydrin spray reagent and a commercial densitometer at 490 nm[5,7]. Proline and hydroxproline are not detectable by that method because of the lack of reactivity. Independent determination was recommended using another spray reagent and scanning at 620 nm. With LPS–FID, all amino acids separated will be detected in one scan. This is because the laser light is absorbed by the TLC plate. Heating is guaranteed regardless of the analytes involved. Most importantly, no spray reagents are needed for LPS–FID.

The calibration curve for amino acids in densitometry is obtained by plotting the peak area *vs.* the square root of the amount and is linear up to 5 μg only. With LPS–FID, peak area is linearly related to the amount of amino acids and the linearity was improved by a factor of three.

The organochlorine pesticides can be detected at the 0.1-μg level with silver nitrate followed by UV photochemical reaction and a fiber-optics densitometer[8]. The linear plot obtained was only over one order of magnitude. With LPS–ECD, both sensitivity and linearity are improved.

Further improvement of this technique relies on having a clean TLC plate, since the signal levels obtained are quite large. Contamination is believed to be the major

source of the background. This includes impurities incorporated during the manufacturing process, exposure in the laboratory atmosphere, and impurities in the developing solvent. The background decreased 62 times after the cleaning procedure for the TLC plates with binder. The plates without binder had a much lower background and thus required only a simple cleaning process. Okumura and co-workers[9,10] have discussed FID background in different types of silica gel quartz rods and found that sintered thin layers have low background. They burned the rod on a flame, which can be moved to scan, and collected ions on the top of the flame. Sintered TLC plates have also been made[11] and have been shown to possess good separation power.

Optimization of the experimental conditions is very important. This includes carrier gas flow-rate, laser scan rate, laser power and pyrolysis cell body temperature. These have not been fully explored in this work. Fast carrier gas flow will provide better resolution, but the sensitivity will decrease due to dilution. Higher laser power is better regardless of the background, for efficient pyrolysis and rapid vaporization. But, here it is limited to below 5 W, beyond which the glass-backed TLC plate may break. The scan rate is limited by the vaporization rate. The volume of the pyrolysis chamber must be kept to a minimum. Temperature control of the pyrolysis cell may be necessary since the analytes may evaporate before pyrolysis and deposit on the cell wall.

Low nanogram detection for LPS–FID and picogram detection for LPS–ECD are certainly possible. In gas chromatography (GC), FID is capable of detecting 10^{-11} g/s of methane with 10^{8} linear range[12], and ECD is capable of detecting as little as 10^{-14} g/s of sulfur hexafluoride with 10^{4} linear range. So, it may be possible to eventually improve on the LOD here. Most compounds can be easily pyrolyzed using laser powers of 5 W. The laser power (3.5 W) used in our experiments may produce a 600–700°C spot (estimated by the brightness of the spot).

Direct identification in TLC is an even more difficult task than quantitation. TLC–LPS should allow one to identify the separated compounds from the gas-phase fragments. For example, by connecting to a gas chromatograph, it is ready for pyrolysis–GC fingerprint identification. It should also be possible for mass spectrometry (MS), GC–MS and GC–Fourier transform IR analysis. For complex mixtures, the additional TLC step provides one more dimension for separation.

The LPS technique is presently limited to silica gel and alumina plates. Reversed-phase plates, cellulose plates and those plates with organic binder are not suitable for FID because of the high organic contents. Also, low boiling point solvents must be used for easy solvent evaporation in LPS–FID to minimize the background. Broad use of TLC–LPS will depend on new types of TLC plates with low background for FID or ECD.

In summary, we have presented a novel quantitation method for TLC based on LPS. No spray reagent is necessary. The analysis time was reduced from several to 20 min. The test samples were chosen for demonstration of the technique. The same principle should apply to all other organic compounds because pyrolysis is a universal mechanism for transferring species to flame ionization or electron-capture detectors.

ACKNOWLEDGEMENTS

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REFERENCES

1 J. G. Kirchner, *Thin-layer Chromatography*, Wiley, New York, 1978.
2 M. E. Coddens, H. T. Butler, S. A. Schuette and C. F. Poole, *LC Mag.*, 1 (1983) 282.
3 H. Kawazumi and E. S. Yeung, *Appl. Spectrosc.*, 42 (1988) 1228.
4 I. I. Chen and M. D. Morris, *Anal. Chem.*, 56 (1984) 19.
5 J. C. Touchstone and J. Sherma, *Densitometer in Thin-layer Chromatography*, Wiley, New York, 1979.
6 S. Laskar and B. Basak, *J. Chromatogr.*, 436 (1988) 341.
7 B. Fried and J. Sherma, *Thin-layer Chromatography*, Marcel Dekker, New York, 2nd ed., 1986.
8 J. Sherma and K. Bloomer, *J. Chromatogr.*, 135 (1977) 235.
9 T. Okumura and T. Kadono, *Bunseki Kagaku (Jap. Anal.)*, 22 (1973) 980.
10 T. Okumura, T. Kadono and A. Iso'o, *J. Chromatogr.*, 108 (1975) 329.
11 T. Okumura, T. Kadono and M. Nakatani, *J. Chromatogr.*, 74 (1972) 73.
12 R. L. Grob, *Modern Practice of Gas Chromatography*, Wiley, New York, 2nd ed., 1985.