This Week's Citation Classic

Blank M L, Schmit I A & Privett O S. Quantitative analysis of lipids by thin-layer chromatography. *J. Amer. Oil Chem. Soc.* **41**:371-6, 1964.
[Hormel Institute, University of Minnesota, Austin, MN]

This paper describes a method for the quantitative analysis of lipids by thin-layer chromatography (TLC) employing photodensitometry of charred spots. Quantitative analysis is performed by comparison of peak areas obtained by scanning each spot of the separated lipids with those of standards. [The *SCI*® indicates that this paper has been cited over 175 times since 1964.]

Merle L. Blank
Oak Ridge Associated Universities
Medical and Health Sciences Division
P.O. Box 117
Oak Ridge, TN 37830

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"This research was done by John Schmit and myself under the direction of Orville Privett at the Hormel Institute, University of Minnesota, Austin, Minnesota. It occurred to us that thin-layer chromatoplates of charred spots of lipids could be analyzed quantitatively by photodensitometry similar to paper chromatograms. It was reasoned further that such a method should be particularly valuable because of its speed and sensitivity compared to column procedures which were the only alternative at the time.

"Because the only available photodensitometer was designed for paper chromatograms, we started out by making instrumental modifications that would allow handling of thin-layer chromatoplates. Initial changes were made with tape, rubber bands, paper, and other sundries; worthwhile alterations were replaced with permanent hardware, which in some cases proved to be temporary. After finally deciding that all useful changes on this particular instrument had been made, we attacked the many variables involved in the thin-layer chromatography itself.

"Variables in adsorbent quality and the preparation of chromatoplates were controlled by making our own. Researchers who have only used commercial, high quality, pre-coated plates are fortunate to have missed the fun involved in the earlier years of thin-layer chromatography when you had to 'roll your own.' All of the time slowly and sometimes surely, other variables such as differences in lipid unsaturation, charring conditions, and Rf values yielded to our efforts. It was found that for best quantitative results, lipid standards similar to the samples in unsaturation should be run on the same chromatoplate. Results usually had a relative error of about 10% which seemed rather high; however in many instances where the amount of sample was limited, no other method of analysis was available. One section of the paper, the use of photodensitometry on x-ray films exposed to radioactive lipids separated by thin-layer chromatography, has not been used as extensively but was the forerunner of modern techniques of radio-TLC analysis. However, the technique of quantitative analysis of lipids by TLC using charring and densitometry is still very widely used.

"Some improvements, designed to minimize the art involved in the basic methodology, have been made since this paper was published. High quality photodensitometers, designed specifically for thin-layer chromatoplates, have been commercially available for the last several years and the method has now been expanded and modified for the analysis of many compounds besides lipids. These developments in instrumentation together with the relative simplicity of the technique have obviously contributed to its widespread use."