

Rapid and sensitive method for the quantitation of non-polar lipids by high-performance thin-layer chromatography and fluorodensitometry

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ABSTRACT

Non-polar lipids were separated by high-performance thin-layer chromatography on silica gel plates and detected by use of a new reagent that induced fluorescence in the separated components. Developed thin-layer plates are dipped into a solution of sulfuric acid-ethanol-hexane (1:35:64, v/v), heated and the lipid classes are quantified by fluorescence densitometry. This technique allowed detection of certain standard lipids at the 5-ng level, is well suited for the rapid and efficient analysis of large numbers of samples and offers distinct advantages over other *in situ* fluorescence inducing methods. The method was successfully applied to the analysis of the non-polar lipids that occur in enzymatically hydrolyzed beef tallow.

INTRODUCTION

Thin-layer chromatography (TLC) has been used extensively for the analysis of lipids [1] and more recently, high-performance TLC (HPTLC) has been supplanting traditional TLC methodology. While extremely useful for lipid separations, broad applications of thin-layer techniques have been limited due to a lack of reliable quantitation of separated components [2]. This limitation was addressed by the development of quantitative methods such as absorbance densitometry of charred [3,4] or reagent-stained [5,6] thin layers, which did allow quantitation, but at the expense of many of the intrinsic advantages of TLC such as speed, sensitivity, minimal sample and plate pretreatment and reproducibility. In addition, absorbance/reflectance densitometry has been shown to yield non-linear calibration curves which have the potential to introduce large errors into a quantitative method [7].

Thin-layer chromatography combined with fluorodensitometric detection obviates many of the difficulties associated with absorbance TLC quantitation and has been used in a number of variations to analyze a broad range of components [8]. A vapor-phase fluorimetric procedure for the *in situ* derivatization of organic compounds on thin-layer plates was developed by Segura and Gotto [9] and this method has been applied to the analysis of lipids [10]. In other work, Schmitz and Assman [11]

made use of a reagent mixture that induced fluorescence of lipids after immersion and subsequent heat treatment of developed TLC plates. While the advantages of fluorescence detection, such as superior reproducibility, lower detection limits and linear response of calibration standards are obtained with these techniques, they are somewhat complex and time consuming to perform and not conducive for use in routine analysis or large scale studies.

In this report, we present a method for the analysis of neutral lipids by HPTLC and fluorodensitometry. We developed this method for the analysis of product streams from immobilized lipase reactors designed for the hydrolysis of beef tallow [12]. Tallow is composed primarily of triacylglycerides, minor amounts of cholesterol and partial glycerides and typical lipase reactor products are mixtures of unreacted triacylglycerides, free fatty acids, diacylglycerides and monoacylglycerides. We required a simple and accurate method of analysis that could quantitate all of the above components found in partially hydrolyzed tallow that would be amenable to the large number of samples required for engineering studies of this process system. The HPTLC method described here appears to satisfy these requirements and would appear to be applicable in the quantitation of many other types of analytes. We have also found, in comparative studies, that this method is more sensitive than those previously reported for the induced fluorescence of lipids [8,11]. Because of this sensitivity the method would appear to be especially suitable for use in studies where only minute amounts of lipid are available for analysis.

EXPERIMENTAL^a

HPTLC plates

Precoated HPTLC plates, 10 × 10 cm, silica gel 60 (Merck, Darmstadt, Germany), without fluorescent indicator, were used for all experiments.

Standards

For method development, commercial lipid-class standards, all with the same fatty acid substitution were used. These were: triolein, oleic acid, 1,2-diolein, 1,3-diolein and monoolein (Nu Chek Prep, Elysian, MN, USA).

Reagents

Hexane and methanol used in this work were HPLC grade (Burdick and Jackson, Muskegon, MI, USA), diethyl ether was analytical-reagent grade (Mallinckrodt, Paris, KT, USA), ethyl alcohol was USP dehydrated 200 proof (Pharmco, Bayonne, NJ, USA) and sulfuric acid was ACS reagent grade (J. T. Baker, Phillipsburg, NJ, USA). The tallow used in this study was edible-grade beef tallow (Ed Miniat, Chicago, IL, USA).

Fluorescence detection

Fluorescence detection of HPTLC-separated components was obtained with a TLC scanner II (Camag, Muttenz, Switzerland) equipped with a mercury lamp.