

AN ACCURATE, HIGH EFFICIENCY RADIOASSAY PROCEDURE FOR
CARBON¹⁴ AND TRITIUM COMPOUNDS SEPARATED BY SILICA
GEL THIN LAYER CHROMATOGRAPHY

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Abstract

The accurate counting of radiolabelled materials after their separation by silica gel thin layer chromatography poses problems because self-absorption limits recovery and counting efficiency. A new method is described that uses hydrofluoric acid to dissolve and deactivate the silica gel and a scintillation solution of toluene:triton X100. This method yields higher efficiencies than other systems and virtually quantitative recoveries of carbon¹⁴ and tritium labelled compounds.

Introduction

Thin layer chromatography (TLC) on silica gel is an efficient means of separating radiolabelled lipids into classes or species. However, difficulty is encountered in the radioassay of carbon¹⁴ and tritium compounds after separation. External scanning of chromatoplates yields low efficiencies and poor resolution. Elution before counting involves additional handling of samples and polar compounds are not quantitatively recovered. The phenomenon of self-absorption limits recovery of low energy radioactivity when the usual scintillation solution containing toluene is added to the TLC fractions. Several approaches have been used to overcome this problem. One technique is to make a gel by adding Cab-O-Sil to a toluene scintillation solution. However, with this method the recovery is not quantitative. Another technique is the use of the polar solvent system, dioxane-naphthalene-water (DNW) to displace the radioactive material from the silica gel.¹ Recoveries using this system are excellent, but the efficiency for tritium is low. Further, the scintillation solution is labile with a maximum shelf life of one month. We have developed a new method

which yields both quantitative recoveries and high efficiencies for carbon¹⁴ and tritium. The principle of the method is that self-absorption, the major problem with other methods cannot occur if the radioactivity is displaced from the TLC absorbant.

General Considerations

The proposed method was developed and compared with other methods by determining the recovery and counting efficiency using carbon¹⁴ and tritium containing lipids. These data will appear in a subsequent publication. Both synthetic and naturally occurring compounds were assayed using the scintillation solution toluene-triton X100 (2:1 volume) containing 5.5g PPO and 125mg dimethyl POPOP/liter. This is a modification of the scintillation solution described by Patterson and Green². A Packard liquid scintillation spectrometer model 3320 using radium²²⁶ as the source of external standardization was used.

The optimal conditions for the assay are as follows: TLC fractions of approximately 1.5cm x 4.5cm dimensions are scraped into glass scintillation vials having caps with polyethylene discs. Water (0.3ml) is added with an automatic pipette. Five to ten vials are usually done in series, thus allowing the water to saturate the silica gel before the HF is added. Then, 0.4ml of HF is added with an automatic pipette having a plastic tip. The vials are swirled by hand until the silica gel is dissolved. These procedures should be carried out under a fume hood. An automatic dispenser is used to add 10ml of toluene:triton X100 scintillation solution. The vials are cooled in the counter for one hour, then shaken and counted. At this time, the scintillation solution is opaque. However, if the samples remain in the counter for 24 hours, they become clear with a small white precipitate on the bottom of the vial. The counting efficiency does not depend on the clarity of the solution. The white precipitate does not contain any radioactivity and is thought to originate from impurities in the triton X100.

The extra steps of this method are not particularly time consuming as 60 samples may be processed per hour, and the advantages of this system are worth the small expendi-

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ture of time. Recoveries range from 98 to 102% for both carbon¹⁴ and tritium. Efficiency for carbon¹⁴ and tritium is 87% and 31% respectively. Also, this scintillation method is not sensitive to quenching of the usual indicators used to visualize the lipids. While the advantages in counting carbon¹⁴ are apparent, particularly when quenching is a problem, the almost doubling of efficiencies for tritium counting are striking and make this the preferable method for counting TLC fractions containing this isotope.

References

1. F. Synder, Anal. Biochem. 9, 183, (1964).
2. M.S. Patterson and R.C. Greene, Anal. Chem. 37, 854, (1965).