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Note

Use of 6-*p*-toluidino-2-naphthalenesulfonic acid to quantitate lipids after thin-layer chromatography

MARJORIE JONES*, ROY W. KEENAN and PAUL HOROWITZ
Department of Biochemistry, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284 (U.S.A.)
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One of the most useful and generally applicable of all of the methods for the detection of lipids on thin-layer chromatography (TLC) is the use of compounds that fluoresce in hydrophobic environments. Several reports[1–3] have described the results obtained using 8-anilinonaphthalene-1-sulfonate (ANS). In this report, we present data which demonstrate that 6-*p*-toluidino-2-naphthalenesulfonic acid (TNS) is considerably more sensitive in the detection of lipids on thin-layer chromatograms than ANS because of its greater quantum yield. We have shown that very small amounts of lipid compounds such as cholesterol can be detected and quantitated using TNS. In addition, several other advantages in using a detection method such as the TNS procedure are presented.

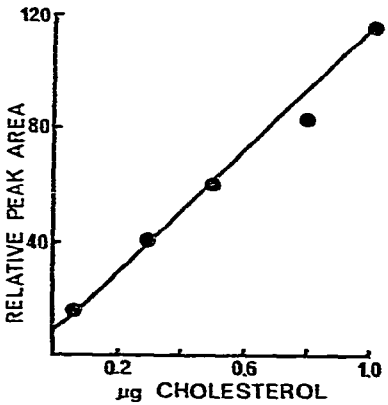
EXPERIMENTAL

The TNS was obtained from Eastman (Rochester, NY, U.S.A.). The working solution was 1 m*M* TNS in 50 m*M* Tris-HCl, pH 7.4, and this solution was stable for several weeks stored in the dark. The reagent required gentle heating to bring the TNS into solution before use. TLC was conducted as usual using HETLC uniplates from Analtech (Newark, DE, U.S.A.) and a solvent of chloroform–methanol–water (65:25:4, v/v/v). After developing the chromatogram, the solvent was evaporated and the plate sprayed with a fine mist of the TNS solution. The lipid spots were observed under a UV lamp (short wave) or scanned using a Helena Quick Scanner equipped with a fluorescent lamp monitoring system. The lipids appear as bright spots on a dark background. For quantitative work, the amount of the lipid was determined by measuring the area of the peak after scanning. Standard cholesterol was obtained from Sigma (St. Louis, MO, U.S.A.). Myelin from rat spinal cord was isolated by the method of Norton and Poduslo[4], and lipids were extracted by the method of Bligh and Dyer[5]. The lipids were stored at −20°C in chloroform. Dolichol was isolated from pig liver as described by Richards and Hemming[6].

RESULTS AND DISCUSSION

fluorescence quantum yield with different lipids; therefore, for quantitative work, standards must be used. For cholesterol, the linear range was 0.06–1.0 μg (Fig. 1). This is also the range detectable by UV light using ANS[1–3]. However, as little as 0.03 μg of cholesterol may be detected with the TNS reagent, using a UV lamp and direct visualization, making the TNS qualitatively more sensitive than the ANS reagent.

TNS was also a non-destructive reagent since it could be removed from the cholesterol after TLC. The UV-visible spots were scraped from the plate, and extracted with chloroform–methanol–water (1:1:0.9) by the procedure of Bligh and Dyer[5]. After re-applying the chloroform extract on a new plate, the extracted cholesterol co-chromatographed with authentic cholesterol and exhibited no fluorescence while the solvent front was fluorescent. Respraying the plate with TNS again showed the position of the cholesterol. The TNS did not significantly change the cholesterol R_F position. The ability to remove TNS from the lipid would allow for the further processing of a valuable material. The lipid could also be removed from the TLC



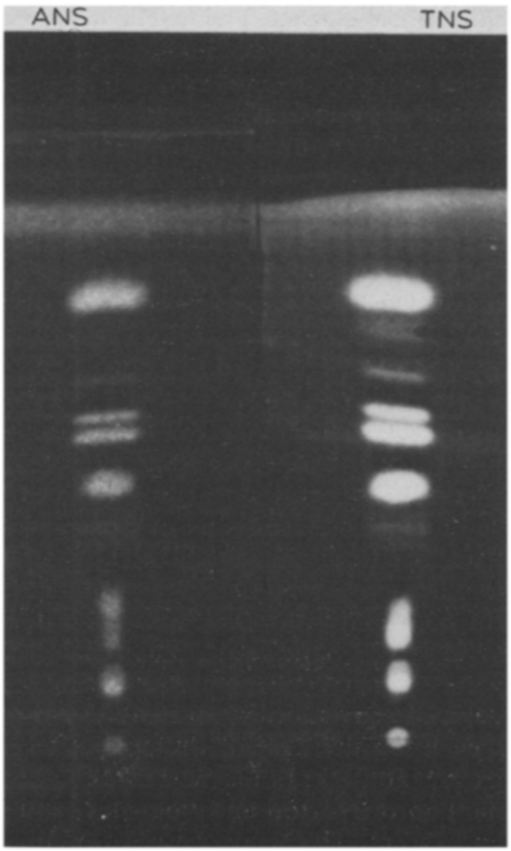


Fig. 1. The relative peak area measured after TNS treatment of cholesterol on TLC. The concentration of cholesterol was varied as indicated.

Fig. 2. Comparison of TNS and ANS with myelin lipids chromatographed on HETLC uniplates. The same amount of lipid extract was applied to each lane. ANS spray is on the left and TNS spray is on the right of the photograph.

plate and radioactivity evaluated, after drying the plate, since the TNS did not interfere with scintillation counting (data not shown).

To compare directly the sensitivity of TNS and ANS, both reagents were made up to the same molar concentration and used, on the same day, to evaluate myelin lipids. The same amount of extract was applied to each lane. The results are shown in Fig. 2. The left side of the plate was sprayed with ANS and the right side with TNS. The photograph (taken using Kodak Tri-X Pan film and UV light) clearly shows that the TNS fluorescence was more intense than that of ANS for each of the myelin lipid spots.

In another another experiment, authentic cholesterol standards were used to quantitate the cholesterol content of rat spinal cord myelin. The myelin lipid extract (10 μl) was applied to a HETLC uniplate and run as before. After evaporation of the solvent, spots of cholesterol standards were applied to another lane of the plate, and the plate was sprayed with TNS and scanned. Comparisons of the areas of the standard peaks indicated that the myelin cholesterol content was 0.81 μg per 10 μl of chloroform extract. The myelin chloroform extract was also evaluated for the cholesterol content by the enzymatic method of Allain *et al.*[7] which required 200 μl of chloroform to be read on the standard curve for this assay. This analysis indicated that 10 μl of chloroform contained 0.65 μg of cholesterol, which is in good agreement with the TNS data. The TNS value, however, was obtained more quickly and required considerably less sample.

In our laboratory, we have also used the TNS spray to detect dolichol in the range 0.04–0.70 nmoles on thin-layer chromatograms. This enabled us to monitor dolichol during the isolation process. TNS is a stable reagent which can be used in a rapid, sensitive, and non-destructive procedure for evaluating lipids on TLC. This procedure should have wide applicability in isolation and quantitation of lipids.

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