Thin-Layer Chromatography of n-Dodecylguanidines

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While attempting to determine the distribution of n-dodecylguanidines in waste water and agricultural run-off it was found that although the colorimetric method of Steller et al. (1) is sensitive and accurate, it is too time consuming for an analytical screening technique. The paper chromatographic techniques described by Curry (2) provided inadequate resolution of the n-dodecylguanidines from other constituents in the environmental samples. Therefore, a thin-layer chromatographic (TLC) method has been developed which requires little sample preparation for the separation and semi-quantitative determination of the n-dodecylguanide moiety of this group of biocides. We wish to report a TLC technique for the separation and detection of any of these biocides in actual environmental water samples.

Experimental:

A standard solution of n-dodecylguanidine acetate (DGA) in acidified methanol (1 ml of conc. HCl added to 1 liter of absolute methanol) equivalent to 1 ug/ul of n-dodecylguanidine was prepared. Four 200 ml aliquots of a lagoon effluent sample[1] were spiked with the DGA standard solution to yield final concentrations of 0, 0.5, 2.0, and 5.0 mg/l, respectively. The samples were then evaporated to dryness at 45±2°C using a rotary evaporator under vacuum, and the residues extracted with three portions of acidified methanol of 4, 2, and 2 ml each. The combined methanolic extracts from each sample were then evaporated to dryness using cotton-filtered air and reconstituted to 1 ml. Ten microliters of each of the samples were applied to a thin-layer plate along with aliquots of the standard DGA solution.

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[1] Water sample was the effluent taken from under ice from the tertiary treatment lagoon of the Green Meadows Sewage Treatment Facility, West Lafayette, Indiana on Jan. 20, 1971. The water characteristics were: pH - 8.5, DO - 32 mg/l, Temp. - 5°C, BOD - 31 mg/l, suspended solids - 74 mg/l (mainly algae), and algal count - 89,000 ASU/ml, 570,000 VSU/ml, 123,000 counts/ml.

The TLC plates were coated with 0.25 mm Absorbasil-1 by conventional techniques and were activated for 1 hour at 100°C before use.

Two chromatographic solvent systems were developed which gave good separation of DGA from other constituents of the sample. The solvent systems were: solvent system A; n-butanol, acetic acid, water (8:1:1) and system B; n-butanol, acetic acid, water (7:1:2). The visualizing agent was iodine vapor.

Discussion:

Solvent A gave a DGA R_f value of 0.6 while Solvent B gave a R_f value of 0.9. Solvent A was considered the better system for this type of sample since the DGA spot was very well defined without tailing and was located away from interfering substances. In separate studies using solvent A and the same TLC techniques, the characteristics of the other three commercial n-dodecylguanidines was studied. The hydrochloride and the normal terephthalate salts were resolved as single spots at R_f 0.6 like the acetate. However, the biphthalate showed two distinct spots with R_f values of 0.55 and 0.65 with weakly reacting material found as a smear between the two spots. The minimum level of detection by the proposed method is 1 microgram of n-dodecylguanidine in the sample applied to the thin-layer plate. The lower sensitivity of this method, when tertiary treatment lagoon water was used, was 0.5 mg/l. A water sample larger than 200 ml introduced sufficient interferring materials to impair the resolution of n-dodecylguanidine.

References:

(1) STELLER, W. A., KLOTSAS, K., KUCHAR, E. J., and NORRIS, M. V., J. Agr. Food Chem. 8, 460 (1960).
(2) CURRY, A. N., J. Agr. Food Chem. 10, 13 (1962).