Experimental

Reagents

N-m-Nitrobenzoyl-N-phenylhydroxylamine. This compound is prepared according to [1] and purified by recrystallisation. A 0.5% solution in isoamyl alcohol is used.

Buffer Solution. 3.4 g of KH_2PO_4 and 25 ml of 0.05 N HCl are made up to 250 ml with water.

Standard Vanadate Solution. An aqueous solution of sodium vanadate is standardized with Mohr salt solution.

Procedure

Take the sample solution with not more than 0.25 mg of V(V) into a separatory funnel, adjust the pH to 2–7, add 5 ml of buffer solution, followed by water to make the volume to 20 ml. Now add the reagent solution (10 ml for each 125 μg of V), shake vigorously and allow the layers to separate. Collect the aqueous layer in a beaker and the organic layer in a dry conical flask containing 2 g of anhydrous Na_2SO_4 . Wash the aqueous layer with 5 ml of isoamyl alcohol, collect the washings in the same flask, transfer the dried extract into a 25 ml measuring flask, wash the conical flask to remove colour adhering to the Na_2SO_4 , add to the main solution and make up to the mark. Measure the optical density against a reagent blank at 435 nm.

Reference

1. Ghosh, N. N., Siddhanta, G.: J. Indian Chem. Soc. **45**, 1049 (1968).

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Quantitative Analysis of Phospholipids by Thin-Layer Chromatography and Evaluation as Molybdenum Blue

Quantitative Analyse von Phospholipiden durch Dünnschicht-Chromatographie und Auswertung als Molybdänblau

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Most of the methods reported for the quantitative analysis of phospholipids depend upon the colorimetric determination of molybdenum blue produced by their P content. Freshly prepared solutions of ascorbic acid or 1-amino-2-naphthol-4-sulphonic acid have been used as reducing agents for the development of the colour. These reagents are unstable and get oxidized very soon; therefore, attempts were made to prepare a stable reagent for the same

purpose. A solution of 2,4-dinitrophenyl hydrazine in HCl has been found to be quite suitable and stable for 10–12 days.

Experimental

Preparation of Reagent. 0.3 g of 2,4-dinitrophenyl-hydrazine was dissolved in minimum amount of conc. HCl and the volume was increased to 100 ml with water.

Method. Two-dimensional thin-layer chromatography was used employing two sets of solvent mixtures, (i) [1] chloroform-methanol-water (65:25:4) and n-butanol-acetic acid-water (3:1:1) as well as (ii) [2] chloroform-methanol-7 N NH_4OH (12:7:1) and chloroform-methanol-7 N NH_4OH (7:12:1). Silica gel layers of 250 μm thickness were prepared. Plates, after drying at room temperature were activated at 120°C for 30 min and cooled for about 20 min before use. The lipid mixture was applied with the help of a micropipette and the plates were developed. Solvents from the plate were allowed to evaporate at room temperature and spots on the plates were located by exposing them in an iodine chamber. Spots of the single phospholipids were identified with the help of authentic samples, spots of equal area and also several blank areas were scraped off and transferred to glass stoppered pyrex test tubes. Each test tube was gently heated over a microburner with 1 ml of 60% perchloric acid until the contents were colourless and thick white fumes evolved (15–20 min). The contents were cooled and the walls of the test tube were washed with 2 ml of distilled water. 1 ml of 5% ammonium molybdate solution was added in each test tube followed by the addition of 1 ml of 2,4-dinitrophenyl hydrazine reagent and the contents shaken well. After 6 hrs silica gel was removed by centrifugation. The supernatant from each test tube was transferred separately to a 25 ml volumetric flask and volume was made up with water. Absorbance of each fraction was measured at 596 nm against a reagent blank. A standard curve was prepared by using Na_2HPO_4 . Sensitivity of the method was increased by using 10 ml volumetric flasks and adjusting the reagents accordingly.

Distribution of phospholipids is expressed as percent of total phosphorous in the lipid mixture which in turn was conveniently determined by applying 50–100 μg of total sample in a blank area left after developing with both solvents and treating that like other spots.

The method was applied to liver lipid extracts of *Calla calla* and ascorbic acid as well as aminonaphthol sulphonic acid were also employed as reducing agents for comparison. It could be shown that the results were in close agreement.

References

1. Angelelli, L., Cavina, G., Moretti, G., Siniscalchi, P.: Farmaco (Pavia) Ed. Prat. **21**, 493 (1966).
2. Skidmore, W. D., Entenman, C.: J. Lipid Res. **3**, 471 (1962).

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