

Es zeigte sich, dass mittels der aufsteigenden papierchromatographischen Trennung besser reproduzierbare Ergebnisse zu erzielen sind.

*Institut für Technische Chemie
der Universität Halle (Deutschland)*

GERHARD SCHWACHULA
FRIEDRICH WOLF

- 1 F. WOLF, A. LOSSE UND G. SCHWACHULA, *Abhandl. Deut. Akad. Wiss. Berlin. Kl. Chem., Geol. Biol.*, No. 1 (1962) 513 (Symposiumsberichte über das 3. Symposium über Gaschromatographie, Buna, im Mai 1961).
- 2 G. SCHWACHULA, *Dissertation*, Halle, 1964.
- 3 W. HUBER, *Mikrochim. Acta*, (1960) 44.
- 4 R. W. MARTIN, *Anal. Chem.*, 21 (1948) 921.

Eingegangen den 1. März 1966

J. Chromatog., 24 (1966) 297-298

A new method of detection of inorganic phosphate on paper

BELEN'SKII AND RISKIN¹ described the formation of a stable alkaline violet lake resulting from mixing of methyl violet and phosphotungstomolybdic acid. AKAMATSU AND IMAI² have described a method for the colorimetric determination of inorganic phosphate based on the reaction of phosphate with methylene violet and ammonium molybdate in diluted hydrochloric acid. A simple and sensitive method of detection of inorganic phosphate on paper based on similar principles and suitable for chromatography is now described.

Reagents

1. Methyl violet, Geigy, 1.0 g, is dissolved in methanol and made up to 100 ml; before use this solution is diluted with diethyl ether 1:3.
2. Ammonium molybdate, 12 g, is dissolved in 150 ml of water by heating, and 35 ml 10 N HCl is added to this solution.

Procedure

The chromatogram is rinsed in solution 1 and after about 30 sec sprayed with solution 2 until the background becomes yellow-green. (The former step can be substituted by intensive spraying.) Inorganic phosphate gives blue spots.

When detecting phosphate esters or polyphosphates (which do not give the reaction mentioned) the dry chromatogram must be previously subjected to acid hydrolysis by commonly known methods. This process can also be realised by spraying with solution 2 followed by 30 min heating at 60-70°. Those conditions were sufficient for ATP, ADP, AMP and pyrophosphate, but the hydrolysis of α - and β -naphthyl phosphate was incomplete.

J. Chromatog., 24 (1966) 298-299

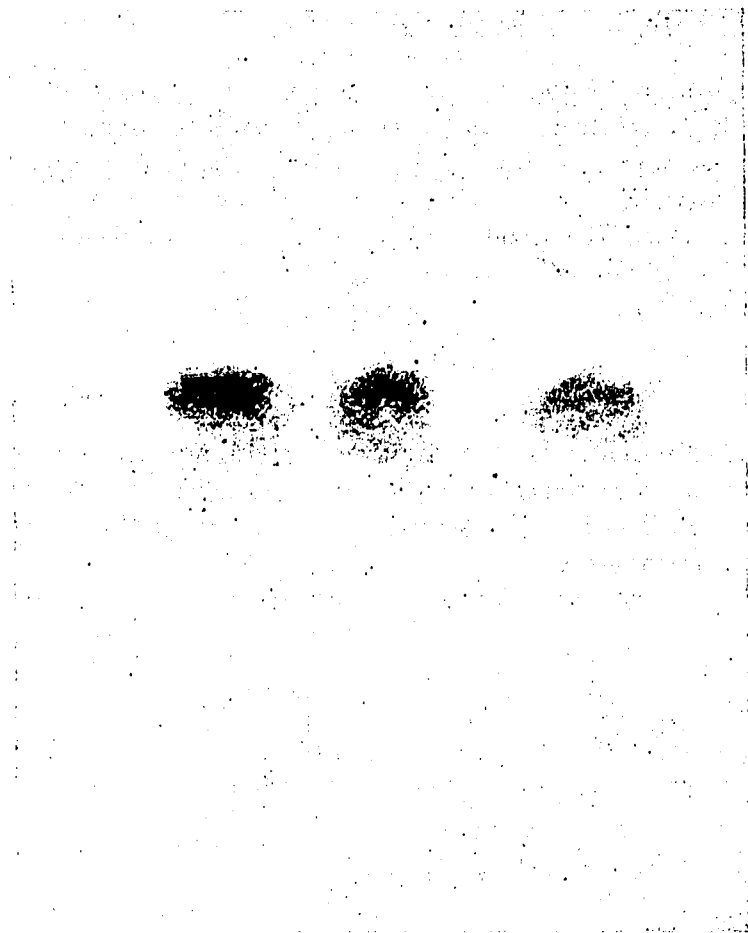


Fig. 1. Detection of inorganic phosphate (from left to right: 0.5, 0.15 and 0.05 μg of P) on chromatogram. System: formic acid 85%–water–dimethyl ketone (15:25:60), ascending development 45 min, paper Ederol 208.

About 0.1 μg of PO_4^{3-} ions can be detected on the chromatograms (Fig. 1). The method is suitable for testing the purity of enzymatic substrates containing phosphate as well as in qualitative analysis, where low quantities of phosphate must be detected.

*First Institute of Medical and Forensic Chemistry,
Charles University, Prague (Czechoslovakia)*

I. HYNIE
J. ŠTĚPÁN
B. VEČERK

1 E. F. BELLEN'SKII AND I. V. RISKIN, *Chimia i Technologia Pigmentov*, Goschimizdat, Moscow, 1949, p. 578.

2 S. AKAMATSU AND K. IMAI, *J. Biochem. (Tokyo)*, 39 (1952) 203; *C.A.*, 46 (1952) 9142 b.

Received January 22nd, 1966