*Journal of Chromatography*, 90 (1974) 390–391


CHROM. 7271

## Note

# Phosphorescence detection method for purine-containing compounds on thin-layer chromatograms

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(First received August 27th, 1973; revised manuscript received November 19th, 1973)

UV irradiation for fluorescence detection of purine compounds following paper or thin-layer chromatography (PC, TLC) is a standard procedure[1]. It is limited by the low quantum yield of fluorescence of most purines. More elaborate techniques utilizing fluorescence or color developing reagents in sprays or dips have also been devised[2–5]. These methods possess high sensitivity and specificity, but they form new products or complexes.

Aaron and Winefordner[6] made an extensive study on the phosphorescence of a number of purine derivatives and Randerath[7] has illustrated that purine derivatives can be detected in minute amounts by photography of the purine phosphorescence afterglow on PEI-cellulose thin-layer chromatograms constantly submerged in liquid nitrogen. Although the latter[7] mentions visual detection of purine phosphorescence, this was not emphasized. This paper discusses the visual sensitivity and simplicity of using purine phosphorescence as a detection tool.

## MATERIALS AND METHODS

Cyclic 3′,5′-AMP, AMP, adenine, and adenosine were purchased from ICN (Irvine, Calif., U.S.A.), while ATP and ADP were obtained from Nutritional Biochemicals (Cleveland, Ohio, U.S.A.).*

Solutions of each of the compounds in glass distilled water were spotted with a microlitre syringe on cellulose chromatogram sheets (Eastman-Kodak, Rochester, N.Y., U.S.A.) and developed with isobutyric acid–water–ammonia (75:25:1).

After allowing the sheets to air dry, phosphorescence was induced by dipping the sheets into a Dewar filled with liquid nitrogen and simultaneously irradiating the plate with short-wave UV light. Illucidation of the different compounds on the plate is best achieved by switching the light off in a darkened room just as the plate is removed from the liquid nitrogen. Presence of the adenyl (purine) compounds was indicated by blue phosphorescence that faded in a few seconds.

* Mention of a company name or proprietary product in this paper does not constitute endorsement by the U.S. Department of Agriculture.

## RESULTS AND DISCUSSION

The natural fluorescence of purine bases is limited by their low quantum yields, hence fluorescence detection is very insensitive[6–9]. The phosphorescence of water–glycerol solutions of nucleic acids at low temperatures was initially observed in 1957 by Steele and Szent-Gyorgyi[10].

In the nanomole range, all of the adenine compounds tested gave excellent phosphorescence responses on cellulose chromatograms. Table I shows the limit of minimum detectability for the particular compounds is approximately 0.5 nanomoles. Thin-layer chromatograms having a large number of spots may have to be taken through the process of inducing phosphorescence several times to insure that the investigator has sufficient time to outline the areas of interest since the phosphorescence decays within a few seconds. Multiple irradiations of the bases do not appear to diminish the initial phosphorescence intensity.

TABLE I
MINIMUM DETECTABILITY LIMITS*

| *Compound* | *Limit (nmoles)* |
|---|---|
| Adenine | 1.00 |
| Adenosine | 0.75 |
| AMP | 0.57 |
| ADP | 0.85 |
| ATP | 0.78 |
| Cyclic 3′,5′-AMP | 0.57 |

* In some cases lower levels could be detected but these were not consistent.

This method should work for most purines and purine derivatives such as guanine, guanosine, guanosine monophosphate, 6-methylpurine, 6-benzylaminopurine, 2,6-diaminopurine, etc. The method would be of little use in studies involving pyrimidine bases since they exhibit little or no phosphorescence[9]. The phosphorescence detection technique should be equally useful in PC work. It is a simple technique unfettered by photographic methods and the necessity of constant submersion of TLC plates in liquid nitrogen. It is also an extremely sensitive non-destructive *in situ* method for purines and disallows interference from fluorescent contaminants since they have short-lived lifetimes of fluorescence (usually $10^{-8}$–$10^{-9}$ sec).

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