Short Communication

Thin-layer chromatography of nucleic acid bases, nucleosides, nucleotides and related compounds

I. Quantitative analysis by direct fluorometry*

Thin-layer chromatography (TLC) is now widely used for the separation of many hydrophilic substances (for reviews see refs. 1–3). For the quantitative analysis of compounds resolved on chromatoplates, the most practical procedure is the direct scanning of spots[1–3]. In this communication we would like to describe our preliminary results concerning direct fluorometry of nucleic acid bases, nucleosides and nucleotides.

Methods

TLC was carried out on purified cellulose MN 300 layers[4], with *n*-propanol–25 % ammonia–water (6:3:1, v/v) in the first, and isopropanol–saturated ammonium sulphate–water (2:79:19, v/v) in the second dimension, using Shandon multiplates-chromatotanks (Shandon Ltd., London) without chamber saturation[4]. This solvent systems has been well established in the chromatographic laboratory of Robapharm for the paper chromatography of nucleo-derivatives.

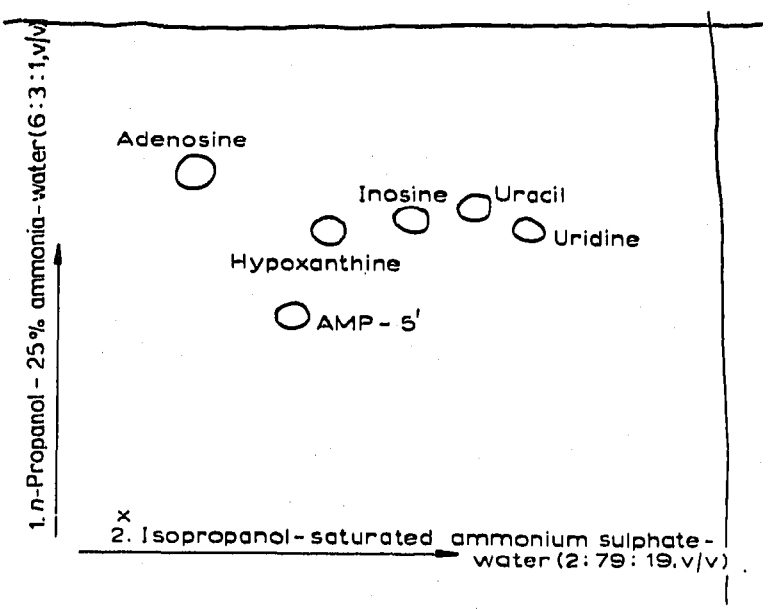

Fig. 1. Separation of some nucleo-derivatives on purified cellulose MN 300 layers[4]. First dimension: *n*-propanol–25% ammonia–water (6:3:1, v/v), second dimension: isopropanol–saturated ammonium sulphate–water (2:79:19, v/v). The chromatography was carried out *without* chamber saturation.

* Preliminary communication.

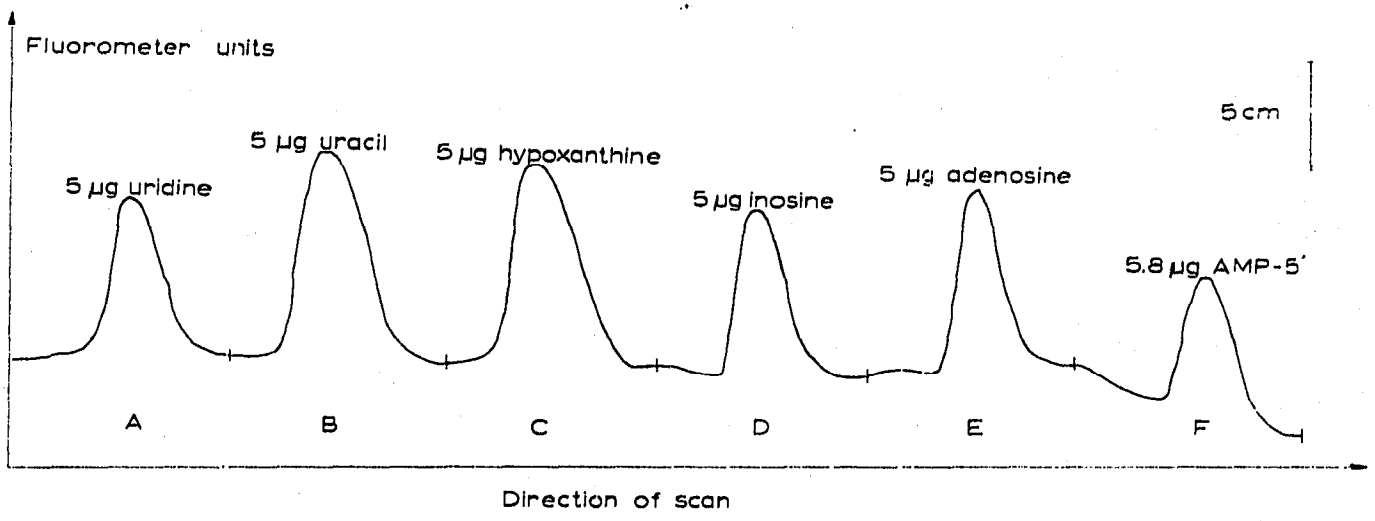

Fig. 2. Typical peaks produced by scanning the spots of adenosine, hypoxanthine, inosine, uracil, uridine (5 μg) and adenosine-5'-monophosphate (5.8 μg) using the CAMAG apparatus (lamp: 110–851; primary filter: 110–810; secondary filter: 110–816; aperture at door: 2 mm; sensitivity: 3 ×).

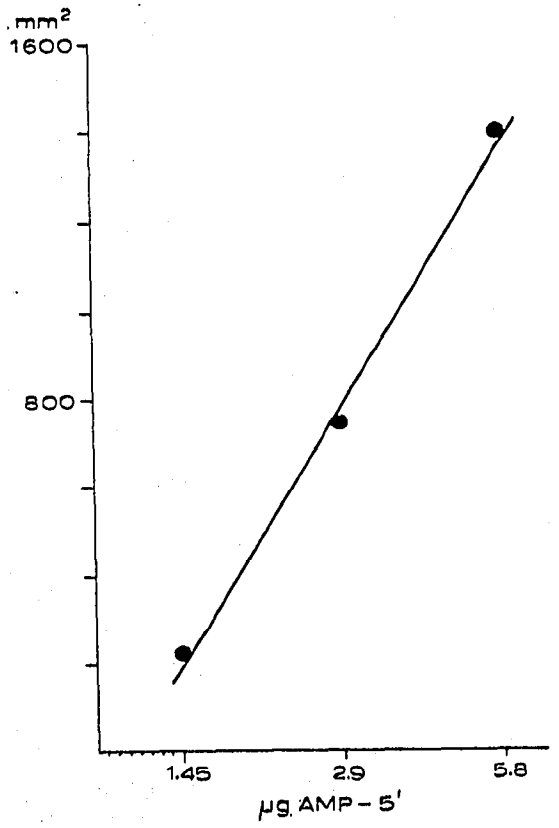

Fig. 3. Peak area plotted against log concentration of adenosine-5'-monophosphate.

Fluorometry was carried out using a Turner fluorometer model 111 fitted with a door for thin-layer plates (supplied by CAMAG*, Muttenz/BL, Switzerland). The experimental conditions are given in Fig. 2. The fluorometer units were recorded by a Varicord model 43 (Photovolt Co., New York).

Results

Fig. 1 shows the separation of some compounds in our chromatographic system. This TLC method has been recently developed in our laboratory and makes the characterisation of about sixty purine and pyrimidine derivatives possible. The procedure will be described in a further publication[4].

Typical curves produced from scanning spots are shown in Fig. 2. Linearity between the area under the peaks and the amount of material applied to the plate exists between 1–5 μg for adenosine-5'-monophosphate (Fig. 3).

The application of this method to the analysis of complex mixtures and full description of the procedure will be given in a subsequent paper**.

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1 E. STAHL (Editor), *Thin-layer Chromatography*, Springer Verlag, Berlin, Göttingen, Heidelberg, 1965.
2 K. RANDERATH, *Dünnschichtchromatographie*, Verlag Chemie, Weinheim, 1965.
3 G. PATAKI, *Dünnschichtchromatographie in der Aminosäure und Peptidchemie*, Walter de Gruyter, Berlin, 1966.
4 G. PATAKI, to be published.

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**Note added in proof*

Since completion of this paper we have found that DNS-, DNP-, and, with some limitations, PTH-derivatives of amino acids can also be quantitatively estimated by direct fluorometry.