Separation and Fluorodensitometric Determination of Some Purine Derivative Drugs by Thin-Layer Chromatography on Starch and Cellulose

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Key Words
Thin-layer chromatography
Starch and cellulose layers
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Summary
Purine derivative drugs, aminophylline, theophylline, xantinolnicotinate, 6-mercaptopurine, 6-thyoguanine, azathioprine, 1-methylxantine, 3-methylxantine, 1-methyluric acid, 1,3-dimethyluric acid, 6-thioxantine, 2-amino-6-methylmercaptopurine were separated by thin-layer chromatography on rice starch and cellulose by three different solvent systems. Conditions for quantitative fluorodensitometric determination of purine derivatives drugs were investigated.

Introduction
Interaction of drugs, as well as interaction of their possible metabolites is a problem in current scientific investigations. Simple, sensitive and rapid methods for qualitative and quantitative determination of drugs and their metabolites are necessary to follow the interaction.

For the separation and determination of some purine derivatives drugs GLC [1, 2], HPLC [3–5], and TLC on silica gel [6] have been used. Our previous investigations showed that rice starch is a useful support for the separation of a range of organic compounds [7–11] and we considered that it might be a useful support for separation of purine derivative drugs and their metabolites by TLC. As a comparison, parallel investigations were also carried out on thin-layers of cellulose.

Experimental
Aminophylline, theophylline, xantinolnicotinate, 6-mercaptopurine, 6-thyoguanine, azathioprine, 1-methylxantine, 3-methylxantine, 1-methyluric acid, 1,3-dimethyluric acid, 6-thioxantine, 2-amino-6-methylmercaptopurine were separated as 1% solutions.

Fig. 1
Chromatogram of purine drugs on thin layers of cellulose with solvent system 2.
1 = aminophylline; 2 = theophylline; 3 = xantinolnicotinate; 4 = 6-mercaptopurine; 5 = 6-thyoguanine; 6 = azathioprine; 7 = 1-methylxantine; 8 = 3-methylxantine; 9 = 1-methyluric acid; 10 = 1,3-dimethyluric acid; 11 = 6-thioxantine; 12 = 2-amino-6-methylmercaptopurine.
A 1% solution of each substance and a 1% solution of the mixture in ammonia (1.0 mol/dm³) were spotted onto the plates by micropipette. Plates were prepared by a standard procedure [7] adding fluorescent indicator F254 (Merck), to improve the identification.

For the separation the following solvent systems were used:
1. Ethyl acetate — methanol — conc. ammonia (50:30:50) (v/v)
2. Tert. butanol — methyl ethyl ketone — conc. ammonia — water (40:30:10:20) (v/v)
3. Ethyl acetate — methanol — conc. ammonia — acetone (20:30:20:30) (v/v)

The best results were obtained with the solvent system 2. On cellulose thin layers, out of a 12 component mixture ten were separated (Fig. 1); on starch thin layers all the components were separated (Fig. 2).

By using starch as the stationary phase and solvent system 2, quantitative fluorodensitometric determination of purine derivatives drugs and their metabolites is possible.

Quantitative fluorodensitometric determination was carried out with a Camag-T-scanner at 254nm. The fluorimetric curves obtained are given in (Fig. 3). Dependence of spot area on concentration for each component of mixture is linear (Fig. 4), and shows that by this method quantitative determination of purine derivatives drugs and their metabolites is possible.

References