Methods for detecting and distinguishing between purines and pyrimidines on paper chromatograms

Purines have been detected on paper chromatograms by a number of chromogenic reagents[1–7]. The methods in references 1–6 also reveal one or more of the commonly-occurring pyrimidine bases as spots similar in appearance to those of purines. These methods, therefore, fail to distinguish between the two types of base with any certainty. A detection procedure which does not appear to reveal pyrimidines is that due to REGUERA AND ASIMOV[7], but, in this laboratory, it has been noted that this procedure fails to detect the purine xanthine. After detection by any of the above methods, the bases are not readily recoverable for further study. This report describes spray procedures which enable purines to be distinguished from pyrimidines and which are highly sensitive for purines including xanthine. After detection, the bases can be readily recovered for spectroscopic and further chromatographic study.

The methods described are based on the following observations. Filter paper sprayed with dilute silver nitrate solution and exposed to light turns brown. This colour development is dependent on an unknown impurity in the paper and on light. At neutral pH, purines either inhibit or modify this colour development to give spots (usually white or yellow) markedly different from those of pyrimidines (mauve). At pH 1.8, purines are revealed as distinctively coloured spots while pyrimidines are not detected. Details of the methods are described below.

Paper chromatography of bases

The following solvents, which do not appreciably elute the impurity necessary for background colour development, are recommended for chromatogram development:

n-butanol saturated with water,
n-butanol–acetic acid–water (12:3:5),
methyl ethyl ketone saturated with water,
ethyl acetate–acetic acid–water (10:3:4, upper phase used).

All filter papers tested (Whatman No. 1, 2, 3 MM, 54 and 540; Schleicher and Schüll No. 598) yielded very similar background colours. The developed chromatograms were dried in an air current at 35° for at least 5 h to remove the solvent completely. The results recorded below were obtained using Whatman No. 1 paper.

Detection procedures and appearance of spots on paper chromatograms

Procedure A. After being sprayed with silver nitrate solution (0.1 %) adjusted to pH 1.8 with formic acid (4 ml per 100 ml of solution), the chromatogram is hung in bright daylight until the background is a light grey-brown. This occurs after 10–30 min depending on light intensity. The chromatogram must not be exposed to direct sunlight. For observing spot appearance, the chromatogram was placed between the illuminating window and the observer.

None of the pyrimidine bases tested (up to 50 μg per spot) were detected by this procedure. These were as follows: thymine, cytosine, uracil, 5-methylcytosine, 1-methylcytosine, 6-methyluracil and 1-methyluracil. All purines tested, except caffeine and theobromine, were located by the spray. The following purines appeared as yellow, orange-yellow or brownish yellow spots: adenine, hypoxanthine, 1-methyl-

guanine, 7-methylguanine, 7-methyladenine, 6-methylaminopurine, 6-(β-hydroxyethyl)aminopurine, 6-benzylaminopurine, 2-methyladenine and kinetin (6-furfurylaminopurine). Spots containing 10 μg or more of certain of these purines, notably the first 5 listed, possessed a pronounced white centre. Xanthine and 6-dimethylaminopurine gave light mauve spots. Guanine in amounts over about 3 μg yielded a white spot but lesser amounts gave an orange-brown spot. The minimum detectable amount of adenine, guanine, hypoxanthine, xanthine, and kinetin on developed chromatograms was 0.3 μg. The lowest detectable amounts of the other purines listed above were not determined precisely, but definite spots were given by 1 μg of all these bases. Procedure A and detection procedures using U.V. light are of similar sensitivity for purines.

Bases can be readily recovered from the sprayed chromatograms. Immediately after the chromatograms have been dried at room temperature, the regions containing the bases can be eluted with 0.1 *N* hydrochloric acid at 25° overnight for determination of absorption spectra. Equal areas of adjacent paper are also eluted to serve as blanks. After determination of spectra, the hydrochloric acid solutions can be evaporated *in vacuo* and the bases subjected to chromatography in other solvents. The above procedure was tested with adenine, hypoxanthine, cytosine, uracil and thymine, the pyrimidines being detected under a U.V. lamp before spraying. In each case, the recovered base and authentic base were chromatographically and spectroscopically indistinguishable. Hence the spray procedure did not appear to degrade either purines or pyrimidines.

To determine whether compounds other than purines and pyrimidines will yield purine-like spots, a range of substances was chromatographed using *n*-butanol–acetic acid–water (12:3:5) as solvent and the developed chromatograms treated according to procedure A. The following compounds in amounts up to 50 μg (the highest tested) were not detected: glucose, fructose, sucrose, *myo*-inositol, quinic, malic, succinic, maleic, fumaric, shikimic and chlorogenic acid, asparagine, glycine, alanine, leucine, agmatine, aspartic acid, benzimidazole, indole, imidazole, cytidine, uridine, deoxycytidine, thymidine, cytidylic acid, uridylic acid and brucine. Arginine, histidine, lysine and chloride (all white spots, sometimes with very faint brown edge) and also citric acid (faint brown spot) were detected but the spots did not closely resemble those derived from purines. A number of substances, however, in amounts of 50 μg (less in some instances) gave spots which closely resembled those of purines. These compounds and the appearance of the spots are listed below: caffeic acid (yellow), ferulic acid (yellow), catechin (yellow), sinapic acid (yellow), gallic acid (pink-brown), indole-3-acetic acid (yellow-brown), deoxyadenosine (yellow-brown), adenosine (yellow-brown), deoxyguanosine (pink-brown), guanosine (pink-brown), adenylic acid (yellow-brown) and guanylic acid (yellow, later pink). The spots derived from the majority of the compounds in the above list were very faint.

Procedure B. The spray used is a 0.1 % silver nitrate solution (pH not adjusted), but in all other respects this procedure is identical with procedure A. The background colour is pink-brown. The following purines appeared as white spots: adenine, guanine, hypoxanthine, xanthine, 2-methyladenine and 7-methyladenine. 6-Methylaminopurine, 6-(β-hydroxyethylamino)purine, kinetin, 1-methylguanine and 7-methylguanine gave yellow spots, while theobromine, 6-dimethylaminopurine and 6-benzylaminopurine were revealed as light pink or orange spots. In amounts of 10 μg and

over, spots of 1- and 7-methylguanine and also 6-methylaminopurine possessed a noticeable white centre. On developed chromatograms the minimum detectable amount of adenine, guanine, hypoxanthine, xanthine and kinetin was 0.3 μg. The lowest detectable amounts of the other purines listed were not determined but would be less than 1 μg. Spots of some of the above purines gradually become tinged with mauve on prolonged exposure in bright light.

All pyrimidines tested (listed under procedure A) were revealed as mauve or grey-mauve spots. The minimum detectable amount of cytosine, uracil and thymine was about 2 μg. Some spots, especially those of uracil, fade and disappear when the chromatograms are dried. Cytosine spots possess a very light mauve centre.

Bases can be recovered after spraying as described under procedure A. Recovered adenine, guanine, thymine, cytosine and uracil showed the correct absorption spectra and R_F values.

Substances other than purines giving purine-like spots in procedure A were also usually revealed as purine-like spots in procedure B. In addition some organic acids, certain amino acids and chloride yielded purine-like spots. Citric acid, quinic acid, malic acid and maleic acid all yielded yellow spots while fumaric acid gave a very faint pink spot. The spots given by these acids resembled those derived from some purines. Other acids (*e.g.* succinic, grey spot; γ-hydroxy-β-methyl crotonic, no reaction), however, did not yield purine-like spots. Procedure B could be useful for distinguishing between certain organic acids. Asparagine and glycine yielded very faint yellow and orange-brown spots respectively. Arginine, lysine, agmatine, histidine and chloride all gave faint white spots.

Succinic and aspartic acid were the only 2 compounds lacking a pyrimidine ring which were found to yield pyrimidine-like spots. Pyrimidine nucleotides and nucleosides also gave mauve spots.

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