23 • AUTORADIOGRAPHY OF PAPER CHROMATOGRAMS

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This chapter, like Chapter 22, fills an important procedural gap in the original manuscript and concerns a method of obtaining a simple picture of a paper chromatogram of a radiopharmaceutical.

Paper chromatograms, thin-layer chromatograms, or other procedures that are used to separate different radiochemical species are usually analyzed by chromatogram scanners or by cutting the paper strips in pieces and counting each piece in a well-scintillation or liquid-scintillation counter. These instruments provide the analyst with quantitative data. At times a rapid, high resolution, qualitative evaluation of the chromatograms is more useful, for example, when the number of radiochemical species is unknown, when it is helpful to know if streaking or decomposition of a radiochemical species is occurring during the development of the chromatogram, when other equipment is not available, and when a simple qualitative result is all that is needed.

Principle of the method

The radioactivity of the radiochemical species distributed on a paper chromatogram can be utilized to expose an ordinary x-ray film. When the x-ray film is placed in direct contact with a chromatogram, the exposure pattern depicts the pattern of distribution of the radiochemical species on the paper. The higher concentrations of radioactivity give the most intense exposure of the film.

Procedure 23-1 is an example of one

Procedure 23-1
Autoradiography of paper chromatograms

1. Mark origin with a lead pencil and spot 10 to 30 μCi of radiopharmaceutical on chromatography paper.
2. Develop chromatogram in the required solvent to give separation between the radiochemical and the expected radionuclidic impurities.
3. Remove chromatogram from solvent and mark solvent front. Dry thoroughly.
4. Mix radionuclide with ink (.02 μCi/μl.) and place small dots of ink-nuclide mixture on three corners of chromatogram. Dry. These marks will permit matching of the autoradiography film with the original chromatogram.
5. In a darkroom, place paper chromatogram and 8-by-10-inch x-ray film in 8-by-10-inch black plastic bag and evacuate, heat, and seal the bag. (Vacuum-heat sealers as used in mammography are ideal; if available the bags may be sealed with tape, but care must be taken to avoid any change in position between the chromatogram and film. Movement during development results in blurred or double exposures. Alternately, the film can be placed in direct contact with the chromatogram in any sort of light-tight container. For example, a film carrying case makes a good exposure chamber.)
6. Exposure times as short as one hour may be used for this level of activity of technetium-99m. If more than one chromatogram is autoradiographed at a time, do not stack the bags during the exposure time or multiple exposures will result.
7. In a darkroom, remove the film by cutting off the end of the bag. Run the film through automatic film processor.

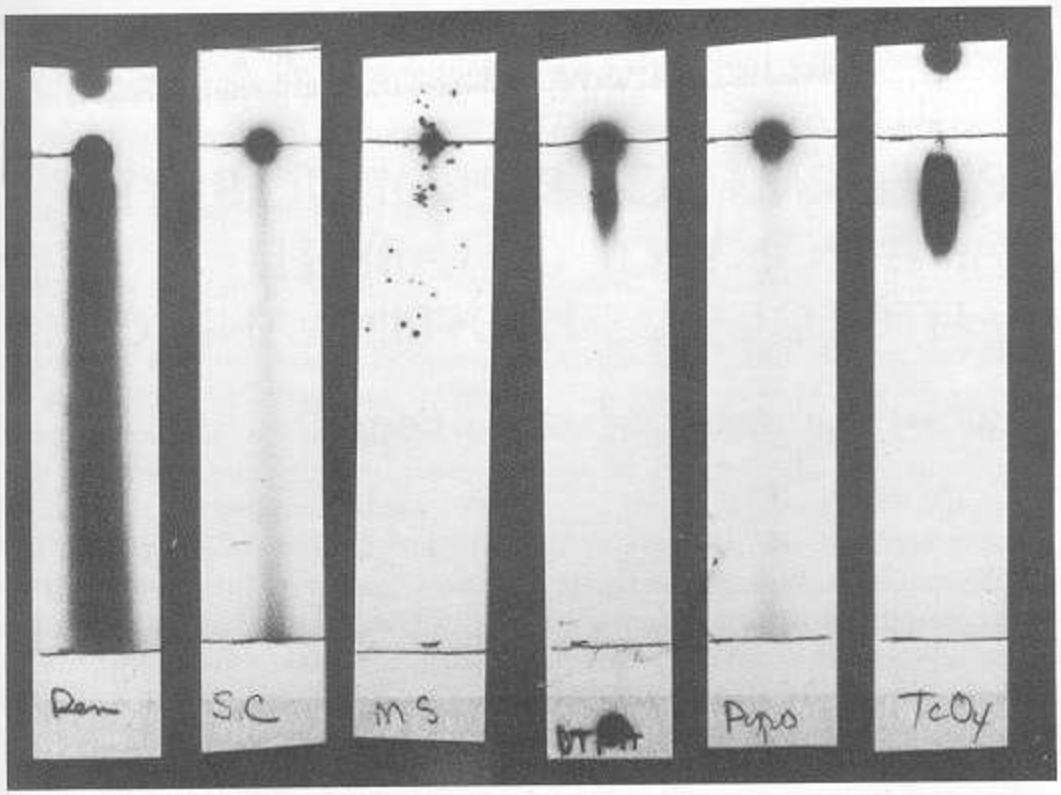

Fig. 23-1. Autoradiogram of several ^{99m}Tc-labeled radiopharmaceuticals (from left to right). *^{99m}Tc-penicillamine.* This radiopharmaceutical shows major streaking of the radioactive species throughout the length of the chromatogram—indicative of chemical decomposition occurring during the development of the chromatogram, probably due to air oxidation of the ^{99m}Tc-complex to free pertechnetate. *^{99m}Tc-sulfur colloid.* The colloidal ^{99m}Tc remains at the origin; minor oxidation occurs during development, and some free $^{99m}TcO_4^-$ is seen at the solvent (from bottom line). *^{99m}Tc-human serum albumin microspheres.* The chromatogram shows no mobile radiochemical species; the small random spots are due to splattering that occurred when the radiopharmaceutical was "spotted" or placed at the origin (top line). *^{99m}Tc-DTPA.* The chromatogram indicated two radiochemical species that are not completely resolved by the paper chromatography. *^{99m}Tc-pyrophosphate.* The chromatogram shows a major chemical species that remains at the origin and a minor species that moves to the solvent front. *$^{99m}TcO_4^-$.* A pure sample of pertechnetate developed in a solvent system in which the $^{99m}TcO_4^-$ moves very slowly.

evaluation of chromatograms. After the film is exposed and developed, it is read by placing it over the chromatograms and visually inspecting it. The matching of the film to the chromatogram is accomplished by matching three dots of radioactive ink on the chromatographic paper with the corresponding three dots of exposure on the film (Fig. 23-1). ^{131}I-rose bengal may be used as the radioactive ink.