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Assay for Pyrimidine Nucleoside Phosphorylases in Tissue Extracts by High-Performance Liquid Chromatography*

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Pyrimidine nucleoside phosphorylases play important roles in the metabolism of nucleic acids and are present in normal and neoplastic tissues. It has been claimed that they are responsible for the catabolism of chemical depot forms of 5-fluorouracil (FU) such as 5′-deoxy-5-fluorouridine (DFUR) and 1-(tetrahydro-2-furanyl)-5-fluorouracil (Tegafur) with real or potential chemotherapeutic usefulness.

A number of assay methods have been proposed for pyrimidine nucleoside phosphorylases. Some are based on the differences in the UV spectra of nucleosides and pyrimidine base in strong alkaline solutions. Most of the assay methods are based on the separation and determination of the phosphorolyzed products, either sugar or base mioeties. Radiolabeled substrates are frequently used in the determination of the bases. Generally, spectrometric method suffer from the inaccuracies and the separation methods from the tedium.

High-performance liquid chromatography was successfully applied for the determination of pyrimidines formed enzymatically from thymidine, uridine, 5-fluorouridine, and 5′-deoxy-5-fluorouridine. The pyrimidines, after extraction with ethyl acetate, are separated by reversed-phase chromatography on μ-Bondapak C-18/Porasil are determined from the heights of chromatographic peaks with UV detection. The method is simple, sensitive and reliable. The limits of detection are 2.5 pmol. 1.0 pmol, and 2.0 pmol for thymine, uracil, and 5-fluorouracil, respectively.

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