5-fluorouracil (5FU) remains one of the most frequently prescribed chemotherapeutic drugs for the treatment of cancers of the gastrointestinal tract, breast, head and neck. To exert its cytotoxic effect against cancer, 5FU must first be anabolized to the nucleotide level. Opposing the activation of 5FU to the level of fluoropyrimidine nucleotides are the enzymes of the pyrimidine degradation pathway. Dihydropyrimidine dehydrogenase (DPD) catalyzes the conversion of 5FU to fluoro-5,6-dihydrouracil which is the initial and rate-limiting step in the catabolism of 5FU. A relationship between the 5FU dose intensity and the therapeutic response, as well as toxicity, has been noted. Patients with a DPD deficiency are unable to degrade 5FU and these patients are at risk of developing severe toxicity after the administration of 5FU (1, 2). Therapeutic drug monitoring of the 5FU levels in plasma requires the fast and unambiguous identification and quantification of 5FU. In this study, we describe a fast and specific method to measure 5FU in plasma with HPLC tandem-mass spectrometry.

**Materials and Methods**

Plasma samples were obtained from colorectal patients receiving bolus administration of 5FU (425 mg/m²) and folinic acid (20 mg/m²). 30 µl of the Internal standard (1,3-15N₂-5FU) was added to 300 µl of plasma and centrifuged over a Microcon YM-30 filter to remove protein. 2 µl of 25% (w/v) HCOOH was added to 70 µl of the deproteinized plasma sample and 50 µl was injected into the HPLC-

**Figure 1.** Comparison between the HPLC-MS/MS method and a HPLC-UV method. R² = 0.98, y = 0.98 x.
Thymidine phosphorylase (TP) catalyses the first step in the degradation of the pyrimidine deoxynucleosides thymidine and deoxyuridine. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disease which is caused by a thymidine phosphorylase deficiency (1). Clinically, MNGIE is characterised by ptosis, progressive external ophthalmoplegia, severe gastrointestinal dysmotility, cachexia, peripheral neuropathy and skeletal myopathy (2). In patients with MNGIE, no or a severely reduced TP activity was detected in leukocytes. A serious drawback of the applied spectrophotometric assay is the fact that the non-specific absorbance of interfering substances of crude tissue extracts hampers the accurate determination of the TP activity.

Determination of thymidine phosphorylase activity by a non-radiochemical assay using reversed-phase high-performance liquid chromatography

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Literature