compounds was 1.2-5% for liquid urines and 2-9% for filter-paper-extracts of the urines. Recoveries of the added metabolites were 97-106% for urine samples and 97-115% for filter-paper-extracts of the urines. Analysis of urine samples from patients with a urea-cycle defect or pyrimidine degradation defect showed an aberrant metabolic profile compared to controls (Table 1).
HPLC with electrospray ionization tandem mass spectrometry allows rapid testing for disorders affecting the pyrimidine de novo pathway. The use of filter-paper strips will facilitate collection, transport and storage of the urine samples.

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Determination of 5-fluorouracil in plasma with HPLC-tandem mass spectrometry

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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.

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Materials and Methods

Plasma samples were obtained from colorectal patients receiving bolus administration of 5FU (425 mg/m^2) and folinic acid (20 mg/m^2). 30 µl of the Internal standard (1,3-$^{15}N_2$-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-

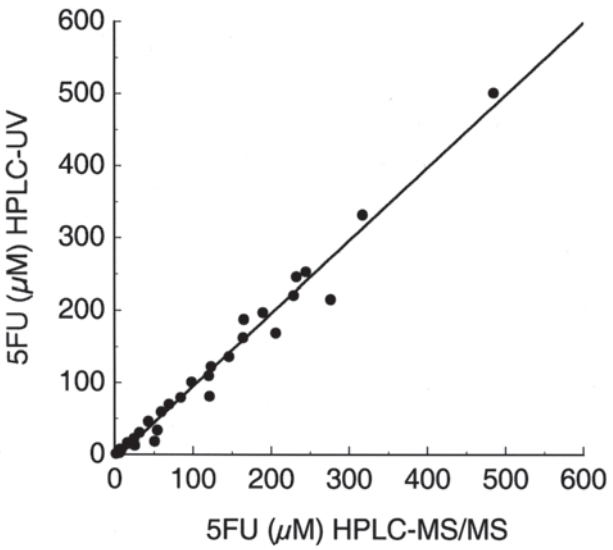

Figure 1. Comparison between the HPLC-MS/MS method and a HPLC-UV method. $R^2 = 0.98$, y = 0.98 x.

MS/MS system. The metabolites were separated on a Phenomenex Aqua analytical column (250 x 4.6 mm, 5 µm particle size), protected by a guard column (SecurityGuard C18 ODS; 4 x 3.0 mm; Phenomenex). Solvent A consisted of 50 mM HCOOH (pH 2.6) and solvent B consisted of methanol. The eluent from 5.8 to 8.0 min was introduced into the mass spectrometer. A Quattro II tandem mass spectrometer (Micromass) was used in the negative Electrospray ionization (ESI) mode and nitrogen was used as the nebulizing gas. Multiple-reaction monitoring was used to detect the metabolites by the specific m/z transition of precursor ion to fragment. Analysis of 5FU levels in plasma was also performed using a reversed-phase HPLC-UV method, as described before (3).

Results

The detection of 5FU and the internal standard 1,3-$^{15}N_2$-5FU was performed using multiple-reaction monitoring with an *m/z* 129→42 and *m/z* 131→43, respectively. The optimal settings of the mass spectrometer for the detection of 5FU and 1,3-$^{15}N_2$-5FU were a cone voltage of 35V and a collision energy of 15 eV. The intra-assay variation and inter-assay variation of plasma with added 5FU (1 µM, 10 µM, 100 µM was less then 6%. Recoveries of the added 5FU (1 µM, 10 µM, 100 µM) were > 97% and the detection limit of 5FU was 0.05 µM. Figure 1 shows that the analysis of the 5FU levels in plasma samples from patients with the HPLC tandem mass spectrometry method and a reversed phase HPLC-UV method yielded comparable results ($r^2 = 0.98$).

Discussion

5FU has a relatively narrow therapeutic index and a strong correlation has been described between exposure to 5FU and both hematological and gastrointestinal toxicity. Despite the many different treatment schedules that exist for 5FU, comparable AUC thresholds have been observed for the onset of severe toxicity. In the case of a deficiency of DPD, profound alterations in the metabolism of 5FU can be expected with an increased likelihood of developing severe toxicity (1, 2). Controlling the AUC of 5FU might therefore be an attractive approach.

In this study we demonstrate that with HPLC-tandem mass spectrometry, 5FU could be measured within 16 min with a detection limit of 0.05 µM, which is at least 6 times more sensitive than the HPLC-UV method (3). The use of stable-isotope-labeled 5FU enabled the correction of the signal for quenching by coeluting compounds, resulting in high recoveries of >97%. The reproducibility of our method is demonstrated by the low intra- and inter-assay variation (< 6%). Thus, the highly specific and sensitive HPLC tandem mass spectrometry method allows the rapid and unambigious analysis of 5FU levels in plasma and could, therefore, be used for therapeutic drug monitoring.

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

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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

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