Rapid determination of gemcitabine in plasma and serum using reversed-phase HPLC

Christian Lanz, Martin Früh, Wolfgang Thormann, Thomas Cerny & Bernhard H Lauterburg

Gemcitabine (2′,2′-difluorodeoxycytidine) is a pyrimidine analog used in the treatment of a variety of solid tumors. After intravenous (i.v.) administration, it is rapidly inactivated to 2′-deoxy-2′,2′-difluorouridine (dFdU). A sensitive analytical method for the quantitation of gemcitabine is required for the assessment of alternative dosage and treatment schemes. A rapid and robust RP-HPLC assay for analysis of gemcitabine in human and animal plasma and serum was developed and validated using 2′-deoxyuridine (dU) and 5-fluoro-2′-deoxyuridine (5FdU) as internal standards. It is based on protein precipitation, the use of an Atlantis dC18 column of 100 mm length (inner diameter, 4.6 mm; particle size, 3 microm) and isocratic elution using a 10 mM phosphate buffer, pH 3.0, followed by isocratic elution with the same buffer containing 3% of ACN. For gemcitabine, RSD values for intraday and interday precision were < 4.4 and 5.3%, respectively, the LOQ was 20 ng/mL, and the assay was linear in the range of 0.020-20 microg/mL with an accuracy of ≥ 89%. The recovery for gemcitabine, dU and 5FdU was 86-98%. The assay was applied to determine gemcitabine levels in plasma samples of patients collected during and shortly after conventional infusion of 25-30 mg/kg body mass (levels: 2.0-18.9 microg/mL) and rats that received lower doses (1.5 mg/kg) via i.v., subcutaneous and oral drug administration (levels: 0.20-2.60 microg/mL). It could also be applied to estimate dFdU levels in human plasma.