HPLC Determination of Active Metabolite of Leflunomide in Plasma

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Leflunomide (LFM), commercial name Arava, produced by Aventis Corp., is a drug used in the treatment of rheumatoid arthritis. Recently, its potential application as immunosuppressant in transplantology has been studied. After oral administration, LFM is rapidly converted into its active metabolite A77 1726. However, A77 1726 causes gastrointestinal irritation, and LFM as a pro-drug is used instead. Pharmacokinetics of A77 1726 significantly differs in particular patients. Due to this fact, monitoring of the level of LFM metabolite in plasma seems to be helpful in the improvement of the treatment with this drug. The aim of this study was to determine A77 1726 concentration in human blood plasma using selective and accurate HPLC method. HPLC analysis was performed using a Spherisorb C8 column. An acetonitrile–methanol–water mixture (40:20:20, v/v/v) served as a mobile phase. Its flow rate was 1.0 mL min⁻¹. Spectrophotometric detection was performed at the wavelength of 280 nm. Column temperature was kept at 70°C. Retention times of A77 1726 and internal standard (IS) were 9.1 and 5.9 min, respectively. 0.5 mL plasma samples containing oxazepam as an IS were mixed with acetonitrile to precipitate proteins. After 10 min of centrifugation at 5°C and 3000 g, 0.5 mL of the supernatant was added to methanol–water mixture. 50 μL of the resulting sample were injected into the column. Within the linear concentration range of 20–200 μg mL⁻¹, recovery was above 90% with the coefficient of variation (CV) 8.2%. Calibration plot was constructed for five experimental points corresponding to five concentration values of the analyte in the extracted samples. The dependence was linear up to 200 μg mL⁻¹; correlation coefficient was better than 0.999. Validation studies confirmed accuracy, high precision, and selectivity of the developed method. The method was applicable to the determination of A77 1726 within the therapeutic concentration range of the analyte. The developed procedure can be recommended in further studies on pharmacokinetics of LFM.