Abstract

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High performance liquid chromatography is one of the most widespread separation analytical techniques. It is used in various medical, pharmaceutical and industrial laboratories. It uses different affinities of the substances in the analysed mixture to the mobile and stationary phases. Mass spectrometry is one of the instrumental techniques by which charged particles are separated in the gas phase based on the mass-to-charge ratio. Nowadays, great emphasis is placed on the fact that the individual analytical methods which are being developed are subsequently validated according to a guideline issued by national or international authorities. Validation is a process that verifies that a method is suitable for its intended use.

In this thesis, the conditions for the determination of warfarin in patient's serum by means of mass spectrometer with LTQ XL linear ion trap were optimized and validated. The optimization of the method was based on the already developed and validated UHPLC-HRMS method in Department of Clinical and Forensic Toxicology, Institute of Clinical Biochemistry and Diagnostics, University Hospital Hradec Králové. In the experimental part, method and conditions of biological material treatment, suitable internal standard were selected. The method was validated in terms of calibration curve, accuracy, precision, matrix effects, recovery and stability.

Protein precipitation with acetonitrile was used to treat the biological material. Citalopram-d6 was used as internal standard. The validation parameters met the criteria according to the EMA provisions.

This method was applied to the measurement of serum warfarin levels in twenty patients and compared with the already validated method in Department of Clinical and Forensic Toxicology, Institute of Clinical Biochemistry and Diagnostics, University

Hospital Hradec Králové. Although the original method clearly showed better accuracy and precision, the new HPLC-LIT-MS method met the validation criteria of the European Medicines Agency (EMA). The results of the measurements differed between measurements at clinically insignificant sub-therapeutic concentrations. This method has shown its value for routine measurements of warfarin in clinical practice.