Abstract

High-performance liquid chromatography belongs nowadays among the most frequently used analytical methods in all fields of the pharmaceutical analysis. It makes both qualitative and quantitative evaluation possible.

Isoniazid is used for the treatment and prevention of tuberculosis. One of the most serious symptoms of toxicity is a neurotoxicity which is connected with the B6 deficiency. The neurotoxicity probably relates to the formation of condensation products isoniazid and vitamins B6 – such as pyridoxal isonikotinoyl hydrazone (PIH). Concrete evidence of the ongoing mechanisms and actions concerning this problem is remains still elusive.

The aim of this thesis was the optimalisation of the chromatography conditions for the separation of 8 analytes (isoniazid, acetylisoniazid, pyridoxal, pyridoxol, pyridoxamine, pyridoxal-5-phosphate, PIH a the internal standard o-108). Thereafter, the isolation of these compounds from plasma samples using precipitation was optimized.

The separation was performed on a LiChroCART 250 × 4 mm I.D., analytical column with Lichrospher 100 RP-18, 5 µm packing, protected with a Purospher RP-18, 5µm guard column.

As the mobile phase A was used aqueous solution of NaH₂PO₄ (0,01 mol/l), with the addition of EDTA (0,001 mol/l) and heptansulphonic acid (0,005 mol/l) with modified pH of the mobile phase using H₃PO₄ to pH 3. Mobile phase B was used methanol. Gradient elution: 0 – 22 min. 10% – 51% B, 22 – 26 min. 51% B, 26 – 27 min. 51% – 10% B, 27 – 40 min. 10% B (v/v). Flowing was 1ml/min. Detection with UV spectrophotometer, wavelenght 260 and 297 nm.

As most optimal isolation method of all 8 analyts was chosen the precipitation of 0.3 ml acetonitril with the addtion of 10 µl perchloric acid.

Recovery factor of individual samples by the means of the chosen precipitation was: isoniazid 97.33%, acetylisoniazid 71.95%, pyridoxal-5-phosphate 76.85%, pyridoxal 79.45%, PIH 81.66%, internal standard 70.61%, pyridoxol 82.43% and pyridoxamine 69.47%.