

High-performance liquid chromatography is one of the most frequently used separation methods for an analysis of drugs in biological material. It is suitable both for quantitative and qualitative drug analysis.

Isoniazid is used for the treatment and a prevention of tuberculosis. Acute isoniazid intoxication is manifested by tonic-clonic convulsions. Vitamine B6 (pyridoxine) is used in clinical practice as an antidote. Although this type of toxicity is considered to be associated with the deficiency of vitamine B6 (or its active form, pyridoxal-5-phosphate - PLP), description of mechanisms and processes, which are responsible for this effect, is still missing.

The aim of this study was to develop suitable chromatographic conditions for monitoring intoxication by isoniazid and detection of potential hydrazone metabolites. These conditions should allow a separation of seven analytes (isoniazid, acetylisoniazid, pyridoxal, pyridoxol, pyridoxal-5-phosphate, and the o-108 - internal standard). Furthermore, the aim was to study possibilities of isolating all those substances from rabbit plasma.

The best chromatographic separation was achieved using the HPLC column LiChroCART 250 × 4 mm I.D., packed with Lichrospher 100 RP-18 (5 µm). The mobile phase was composed of a buffer - component A (NaH₂PO₄ 0.01 mol/l, with the addition of 0.001 mol/l EDTA and 0.005 mol/l 1-hexansulfonic acid, pH 3.0 - modified with 30% H₃PO₄) and methanol - component B, using a gradient: 0 - 18 min. 15 - 55% B, 18 - 21 min. 55% B, 21 - 22 min. 55 - 15% B, 22 - 29 min.

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15% B (v/v). The flow rate was 1 ml/min, the detection was performed at 260 and 294 nm. The SPE method and the precipitation procedure were tested as sample pretreatment techniques. In this case the precipitation procedure appears to be more convenient. Precipitation using methanol was proved to be suitable isolation of isoniazid, acetylisoniazid, pyridoxol, PIH and o-108. The precipitation employing addition of perchloric acid prior to methanol proved the best results for pyridoxal and pyridoxal-5-phosphate. The outcomes of this work will be used for further optimization of extraction methods. This work represents a pilot study aimed at the development of HPLC analysis for monitoring of isoniazid intoxication and detection of new hydrazone metabolites, which have not been described yet.