1. ABSTRACT

High performance liquid chromatography (HPLC) is one of the most frequently used analytical techniques for the analysis of drugs.

Although iron is a vital element, excessive amounts in the body are highly toxic. The search for highly selective and effective iron chelating agents has been mainly inspired by the need to mobilize iron from tissues that are chronically overloaded with iron. However, recent investigations focused on the possibility to use iron chelators for the treatment of many other pathologies.

Salicylaldehyde isonicotinoyl hydrazone (SIH), a biocompatible iron chelator derived from aroylhydrazone, is under extensive investigation as a promising drug candidate. Besides ability to bind iron, it shows interesting pharmacological effects: antioxidative, antiproliferative, cardioprotective, antimalarical and antimicrobic.

The aim of this study was to develop optimal HPLC conditions for the separation of SIH and its potential metabolites (isoniazide, acetylisoniazide, salicylaldehyde) and to apply the method to the study focused on the isolation of analytes from rabbit urine using SPE.

The best chromatographic analysis was achieved on a HPLC column (Phenomenex 250× 4.6 mm I. D.) packed with Prodigy 5u ODS3 100A (5 μm) as a stationary phase. The mobile phase was composed of methanol : acetate buffer (CH_3COONH_4 0.05 mol/L; pH 7.0 – adjusted with sodium hydroxide diluted RS). The following gradient was used: 0. min. 7:93, 8. min. 7:93, 13. min. 58:42, 30. min. 58:42, 31. min. 7:93, 50. min. 7:93 (v/v). A flow rate was 0.9 ml/min. and the UV detector was set at 260 nm from 0. to 10. min. and at 288 nm from 10. min.

Employing SPE extraction, following recoveries of the analytes were achieved: SIH 73.51%; isoniazid 55.06%; acetylisoniazid 55.38%; salicylaldehyde 97.03% and o-108 (internal standard) 88.31%.