

ABSTRACT

The aim of this diploma thesis was the development of a screening method for analysis of 17 benzodiazepines in urine samples using ultra high-performance liquid chromatography with tandem mass spectrometric detection. The partial task was to optimize the conditions for the enzymatic hydrolysis of benzodiazepine glucuronides present in urine using design of experiments (DOE).

The optimized chromatographic system consisted of a Zorbax Eclipse Plus Phenyl-Hexyl RRHD column (100 × 2.1 mm, 1.8 μm) and mobile phase consisting of water with 0.1 % acetic acid (component A) and acetonitrile with 0.1 % acetic acid (component B) in various ratios according to the gradient program. Flow rate was 0.2 ml/min, column temperature was 40 °C, and total analysis time was 12 min. Calibration curves for all analytes were measured under optimized conditions in methanol and urine.

After optimal detection conditions for oxazepam-glucuronide were found, oxazepam glucuronide was hydrolysed using β-glucuronidase from the abalone to confirm the functionality of the enzyme within the pilot experiment. Optimization of enzymatic hydrolysis conditions *via* 27 experiments proposed by program Minitab 16 using the Box-Behnken design will be realized later.