

## Abstract

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Title of thesis: HPLC of bioactive compounds

This diploma thesis is focused on finding conditions of HPLC analysis of midazolam and its two metabolites – 1-hydroxymidazolam and 4-hydroxymidazolam, on method validation and on finding an appropriate internal standard.

The analysis was carried out under reversed-phase conditions, the chosen column was POROSHELL 120 EC-C18, 3.0 X 100 NM, 2.7  $\mu\text{m}$ , and a mixture of acetonitrile and ammonium acetate buffer with pH 4.5 in a ratio of 35/65 has been proven to be effective mobile phase. Detection was carried out using the diode array detector with wavelength values 223 nm and 272 nm. The column was thermostated at 25 °C, the analysis time was 10 minutes. Flow rate of mobile phase was 0.700 ml/min. Diazepam was found as a suitable internal standard.

The method was validated only for 1-hydroxymidazolam and 4-hydroxymidazolam determination in rat plasma due to a submitter's request. Within the analytic method validation the following parameters were tested: precision, accuracy, lower limit of quantitation, linearity and selectivity.