

## ABSTRACT

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Title of Diploma Thesis: Micellar electrokinetic chromatography for the separation and assay of indomethacin and its degradations products

A new method of micellar electrokinetic chromatography (MEKC) for analysis of indomethacin and its degradation products, 4-chlorbenzoic acid (4CHBA) and 5-methoxy-2methyl-3-indolylacetic acid (MMIAA), was developed and optimized. The separation was carried out in uncoated fused silica capillary (with i.d. 75  $\mu\text{m}$ , total length 33 cm and effective length 24,5 cm) with applied voltage 15 kV and temperature of capillary 25°C. Wave length for UV detection was 224 nm and 234 nm. The optimized background electrolyte contained: 10mM phosphate buffer, 60mM SDS (pH 7,0) and 10% (v/v) of methanol. Internal standard was 1-naftylacetic acid. Large volume sample stacking method was used for sensitivity enhancement. The mixture of standards was injected hydrodynamically at 100 mBar for 10 s (24% of capillary total length). Voltage -7,5 kV was applied for stacking of the analytes. Polarity was switched to +15 kV (time of switching 1,5 min), when 95% of original voltage was reached. Analysis of standards took place in time shorter than 7,5 min under optimal conditions. The calibration curves for the impurities were linear in the range 0,125  $\mu\text{g/ml}$  do 10  $\mu\text{g/ml}$  with correlation coefficients  $\leq 0,9992$ . The RSD of migration times and areas ratio ranged between 0,46% and 0,90% (n=6). The limit of detection was 38 ng/ml (MMIAA) and 36 ng/ml (4CHBA). The repeatability of the method (RSD, n=6) was 6,90% (MMIAA) and 7,11% (4CHBA). Preliminary assay of MMIAA and 4CHBA in pharmaceutical product Indobene gel was also carried out.