Migration behaviour and separation of tramadol metabolites and diastereomeric separation of tramadol glucuronides by capillary electrophoresis

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"...The pK_a values of TR and its metabolites were estimated with the Pallas program (Compudrug)..."

Abstract

Capillary electrophoresis with UV detection was used to separate tramadol (TR), a centrally acting analgesic, and its five phase I (M1, M2, M3, M4, M5) and three phase II metabolites (glucuronides of M1, M4 and M5). Several factors were evaluated in optimisation of the separation: pH and composition of the background electrolyte and the influence of a micellar modifier, sodium dodecyl sulfate. Baseline separation of TR and all the analytes was obtained with use of 65 mM tetraborate electrolyte solution at pH 10.65. The lowest concentrations of the analytes that could be detected were below 1 M for the *O*-methylated, below 2 M for the phenolic and ca. 7 M for the glucuronide metabolites. The suitability of the method for screening of real samples was tested with an authentic urine sample collected after a single oral dose (50 mg) of TR. After purification and five-fold concentration of the sample (solid-phase extraction with Oasis MCX cartridges), the parent drug TR and its metabolites M1, M1G, M5 and M5G were easily detected, in comparison with standards, in an interference-free area of the electropherogram. Diastereomeric separation of TR glucuronides in in vitro samples was achieved with 10 mM ammonium acetate–100 mM formic acid electrolyte solution at pH 2.75 and with basic micellar 25 mM tetraborate–70 mM SDS electrolyte solution at pH 10.45. Both separations showed that glucuronidation in vitro produces glucuronide diastereomers in different amounts. The authentic TR urine sample was also analysed by micellar method, but unambiguous identification of the glucuronide diastereomers was not achieved owing to many interferences.

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