Simultaneous Determination of Promethazine and its Metabolites by Improved Capillary Electrophoresis Coupled with Electrochemiluminescence

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Promethazine sulfoxide (PMZSO), dioxopromethazine (DOPMZ) and demethylpromethazine (DMPMZ) are all the main metabolites of promethazine (PMZ) in vivo. DMPMZ has only one methyl less than PMZ. The structural difference between PMZ and DMPMZ is too small to separate them by conventional capillary electrophoresis (CE). However, the separation efficiency of PMZ and DMPMZ can be improved by adjusting the composition of separation buffer. Combined with electrochemiluminescence (CEL) detection technology, a simultaneous determination method of PMZ, PMZSO, DOPMZ and DMPMZ by improved CE coupled with end-column CEL was established. The parameters about CE separation and CEL detection were investigated in detail. The optimum experimental conditions were detection potential 1.20 V (vs. Ag/AgCl), 40 mmol/L of phosphate buffer solution (pH 6.5) containing 6 mmol/L Ru(bpy)₃²⁺ in CEL detection cell, separation buffer of 21.5% iso-propyl alcohol aqueous solution (v/v) containing 20 mmol/L phosphate (pH 5.0), separation voltage of 14 kV, sample injection time of 7 s and sample injection voltage of 12 kV. The limits of detection (3σ) of this method were 7.3×10⁻⁷ mg/mL for PMZ, 8.5×10⁻⁶ mg/mL for PMZSO, 1.5×10⁻⁶ mg/mL for DOPMZ and 4.7×10⁻⁶ mg/mL for DMPMZ. The relative standard deviations were less than 2.8% for ECL intensity and less than 1.1% for migration time. This method was successfully utilized to simultaneously determine PMZ, PMZSO, DOPMZ and DMPMZ in whole urine sample of pet dog.

Keywords: Improved capillary electrophoresis; Electrochemiluminescence; Promethazine; Metabolite; Urine sample

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