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Ionic Liquids Assisted Micellar Electrokinetic Chromatography of Urine Catecholamine Metabolites for the Investigation of Neuroblastoma

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Summary

Neuroblastoma (NBL) is a malignant tumor originating from neural tube cells and is the most common cancer diagnosed in infants. Nearly 50% of its cases occur in children under 2 years of age. NBL is characterized by the increased production of catecholamines (CAs) and their metabolites therefore the quantification of CAs is important for the diagnosis especially the measurement of urinary VMA, HVA and also metoxycatecholamines such as metanephrine (M) and normetanephrine (NM). Capillary electrophoresis (CE) was used in this study due to its number of advantages such as high resolution, speed and low consumption of reagents. However, due to poorer detection limits and lower repeatability, compared to chromatographic techniques, the addition of ionic liquids (ILs) to the separation buffer was investigated to solve these problems. Among the 12 tested imidazolium-based ILs with various substituents in position 1 and various anions, 1-hexyl-3-methylimidazolium chloride turned out to be the most optimal. Literature data confirmed that coating the capillary wall with a cationic layer can increase its surface stability, thereby improving the repeatability of the separation process [1,2]. In this study, micellar electrokinetic chromatography (MEKC) with separation buffer composed of 5 mM sodium tetraborate, 150 mM boric acid, and 50 mM SDS and MeOH (15%, v/v) (apparent pH 7.27) was employed [3]. The isolation of analytes of interest from urine samples was performed by using solid-phase extraction with hydrophilic–lipophilic-balanced columns and methanol as eluent. The obtained results demonstrated that HVA and VMA are easily extracted at pH of 5.5, while a sample pH of 9.0 facilitated the extraction of M and NM. The validation data confirmed the method's linearity ($R2 > 0.996$) for all analytes within the range of 0.25–10 µg/mL. The applicability of the optimized SPE-MEKC-UV method was confirmed by employing it to quantify clinically relevant CAs in real urine samples from pediatric neuroblastoma patients.

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