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Chiral HPLC method optimization for enantioseparation of 2-methylmethcathinone

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Abstract

Background: 2-Methylmethcathinone (2-MMC) is a chiral new psychoactive substance whose enantiomers may differ in pharmacological and toxicological behavior. Reliable enantioselective analysis is therefore required for forensic and environmental applications. Recent work shows that polysaccharide-based chiral stationary phases (CSPs) are broadly effective for cathinones [1]. **Objective:** This study aims to develop and optimize a rapid, robust high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method achieving baseline separation [resolution (Rs) $\geq$ 1.5] of 2-MMC enantiomers. **Methods:** A two-stage workflow was used. First, a screening compared a Lux AMP 3 µm (150 × 4.6 mm) under ammonium bicarbonate (pH 11) with methanol or acetonitrile (isocratic/gradient) against a Lux Amylose-1 3 µm (150 × 4.6 mm) operated in normal-phase (n-hexane/isopropanol). Ultraviolet detection was set at 254 nm. Performance criteria included Rs and run time. **Results:** The Lux AMP configuration yielded limited enantioresolution across tested conditions (maximum Rs $\approx$ 1.11). In contrast, Lux Amylose-1, with n-hexane/isopropanol as mobile phase, produced baseline separation of 2-MMC enantiomers (Rs > 1.5) with short analysis times and consistent retention, providing suitable peak shape and repeatability. These outcomes align with literature showing high success rates of amylose/cellulose CSPs for cathinones under normal-phase and polar-organic modes [2,3]. **Conclusions:** The screening-to-optimization strategy delivered a fast enantioselective HPLC-UV method for 2-MMC. Beyond analytical separation, the method provides a robust platform to develop and adapt enantioselective procedures for biological samples (*e.g.*, oral fluid, blood, urine), enabling enantiomer-resolved quantification in clinical and forensic toxicology.

Keywords: 2-methylmethcathinone; chiral HPLC; enantioseparation

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References

1. Kobidze, G. et al. Simultaneous enantioselective determination of 2-, 3-, and 4-methylmethcathinones; their isomers; and major phase-1 metabolites in oral fluid of drug abusers using enantioselective high-performance liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* **2025**, *417*, 3231-3243, doi: 10.1007/s00216-025-05838-8.
2. Seibert, E. et al. Exploring a Lux® i-Amylose-3 column in normal phase and polar-organic mode for chiral separation of cathinone derivatives and pyrovalerones using high-performance liquid chromatography. *Chirality* **2024**, *36*, e23679, doi:10.1002/chir.23679.
3. Hägele, J.S. et al. A Simple HPLC–UV approach for rapid enantioseparation of cathinones, pyrovalerones and other novel psychoactive substances on a 2.5-µm cellulose tris-(3,5-dimethylphenyl-carbamate) column. *Chromatographia* **2020**, *83*, 321-329, doi: 10.1007/s10337-020-03860-9.